



# **PROGRAM and PROCEEDINGS**

**of the 95<sup>th</sup> Annual Meeting  
December 7, 8 and 9, 2014**

**Marriott, Downtown Magnificent Mile  
Chicago, Illinois**

**Robert P. Ellis, Executive Editor**

<http://www.cvmbs.colostate.edu/mip/crwad/>

**The 95<sup>th</sup> Annual Meeting of the  
CRWAD is dedicated to**

**Dr. Donald C. Robertson**

**Proceedings Distributed by CRWAD**

# CRWAD 95<sup>th</sup> ANNUAL MEETING-2014

December 7 – 9, 2014

**All attendees and presenters are required to wear their name badges at all times.**

## **Registration - 5<sup>th</sup> Floor Registration Booth**

Sunday 10 AM - 5:30 PM

Monday 7:00 AM - Noon, 2 - 5 PM

Tuesday 8 - 11 AM

## **Researchers Reception - Welcome all attendees. Casual Wear**

Sunday, December 7, 6-8 PM – Grand Ballroom Salon III - 7<sup>th</sup> Floor

Introduction of CRWAD Officers and Dedicatee, Poster Session I

**Student Reception**—Students & invited guests-5:00 PM–5:45PM, Los Angeles/Miami Rm, 5<sup>th</sup> Fl

## **Business Meeting - Chicago Ballroom A/B/C/D 5<sup>th</sup> Floor**

11:45 AM - 12:30 PM Tuesday, December 9

Dedication of the meeting, Introduction of New Members, Grad Student Awards

New member applicants and students entered in competition are invited and encouraged to attend.

**Speaker Ready Room is: Streeterville Room (2<sup>nd</sup> floor) - Sunday, Dec. 7 - Monday, Dec. 8**

Marriott Hotel	Monday AM 8:00 - 11:30 Room Abstract Nos.	Monday PM 1:30 - 4:30 Room Abstracts Nos.	Tuesday AM 8:00 - 11:30 Room Abstracts Nos.
Bacterial Pathogenesis	Avenue Ballroom 001 – 009	Avenue Ballroom 010 – 013	Avenue Ballroom 014 – 019
Biosafety and Biosecurity		Denver/Houston 021 – 030	
Companion Animal Epidemiology	Michigan/Michigan State 031 – 041		
Ecology and Management of Foodborne Agents		Salon E 043 – 053	Salon E 054 – 061
Epidemiology and Animal Health Economics	Salons A/B/C/D 062 – 072	Salons A/B/C/D 073 – 083	Salons A/B/C/D 084 – 093
Immunology	Salons F/G/H 095 – 106	Salons F/G/H 107 – 115	Salons F/G/H 116 – 123
Pathobiology of Enteric and Foodborne Pathogens	Los Angeles/Miami 125 – 135		
Respiratory Diseases	Indiana/Iowa 137 – 148	Indiana/Iowa 149 – 152	
Vector-Borne and Parasitic Diseases	Denver/Houston 153 – 160		
Viral Pathogenesis		Los Angeles/Miami 161 – 170	Los Angeles/Miami 172 – 181
Posters* in Grand Ballroom	Salon III-7 <sup>th</sup> Floor Sun. 6:30 - 8 PM	Salon III-7 <sup>th</sup> Floor Mon. 5 - 6:30 PM	

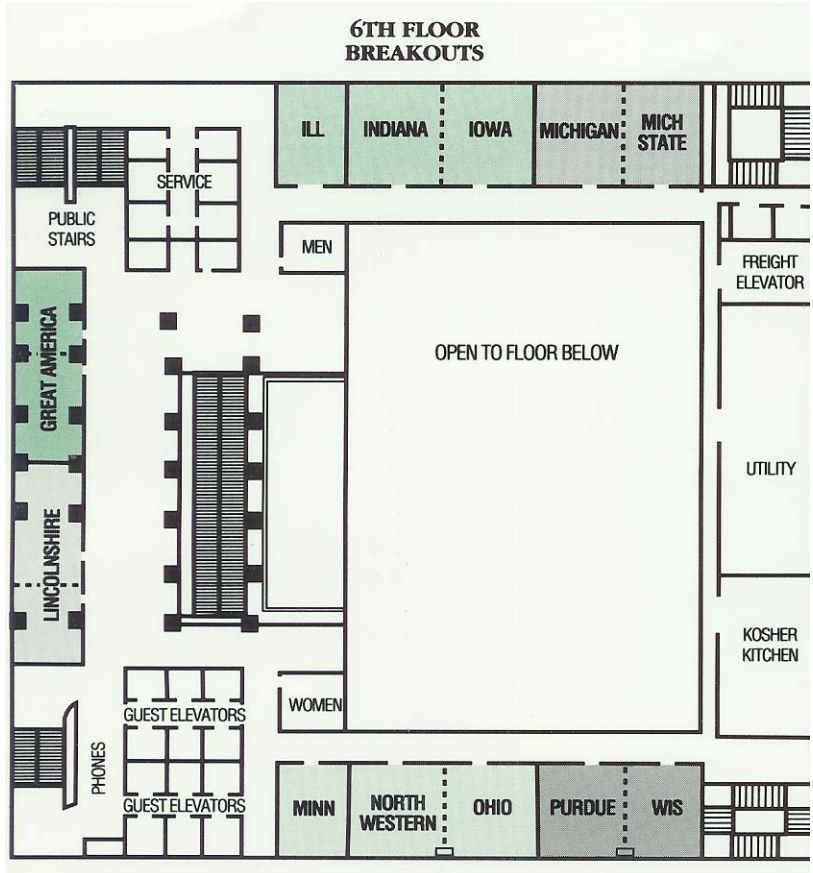
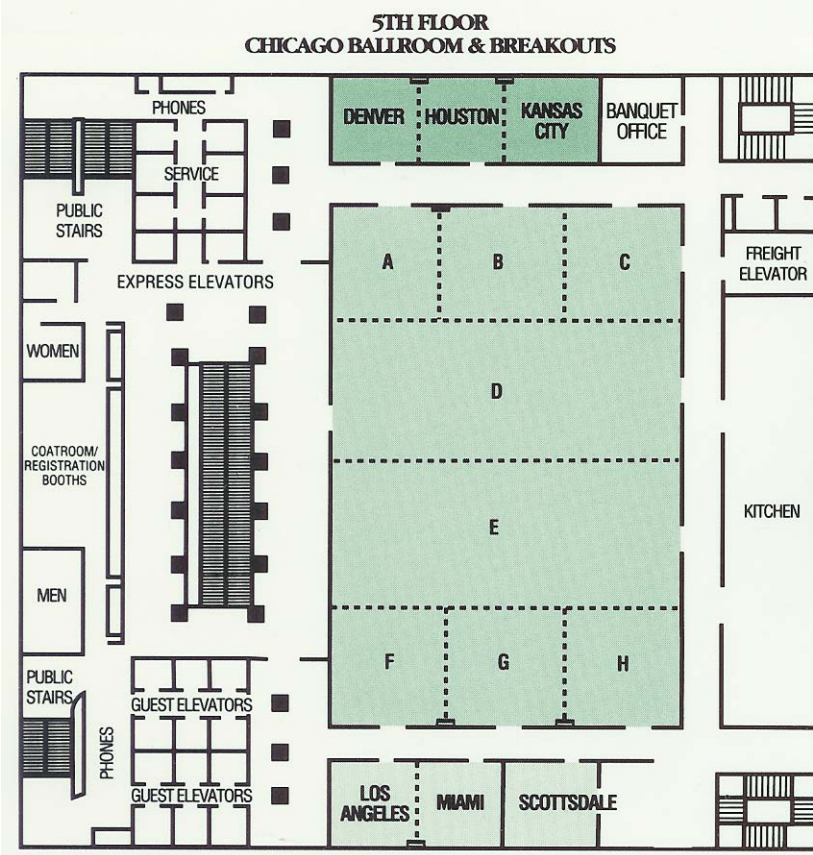
**\*SUNDAY POSTER PRESENTERS:** Poster boards will be available for poster assembly by 4 PM Sunday. Posters for the Bacterial Pathogenesis, Companion Animal Epidemiology, Epidemiology and Animal Health Economics, and Pathobiology of Enteric and Foodborne Pathogens Sections will be presented Sunday from 6:30-8:00 PM. Please remove your posters by 10:00 AM Monday.

**\*MONDAY POSTER PRESENTERS:** Poster boards will be available for poster assembly by noon Monday. Posters for the Ecology and Management of Foodborne Agents, Immunology, Respiratory Diseases, Vector-Borne and Parasitic Diseases, and Virology Sections will be presented Monday from 5:00-6:30 PM. Please remove your posters immediately upon completion of Poster Session II, by 6:30 PM.

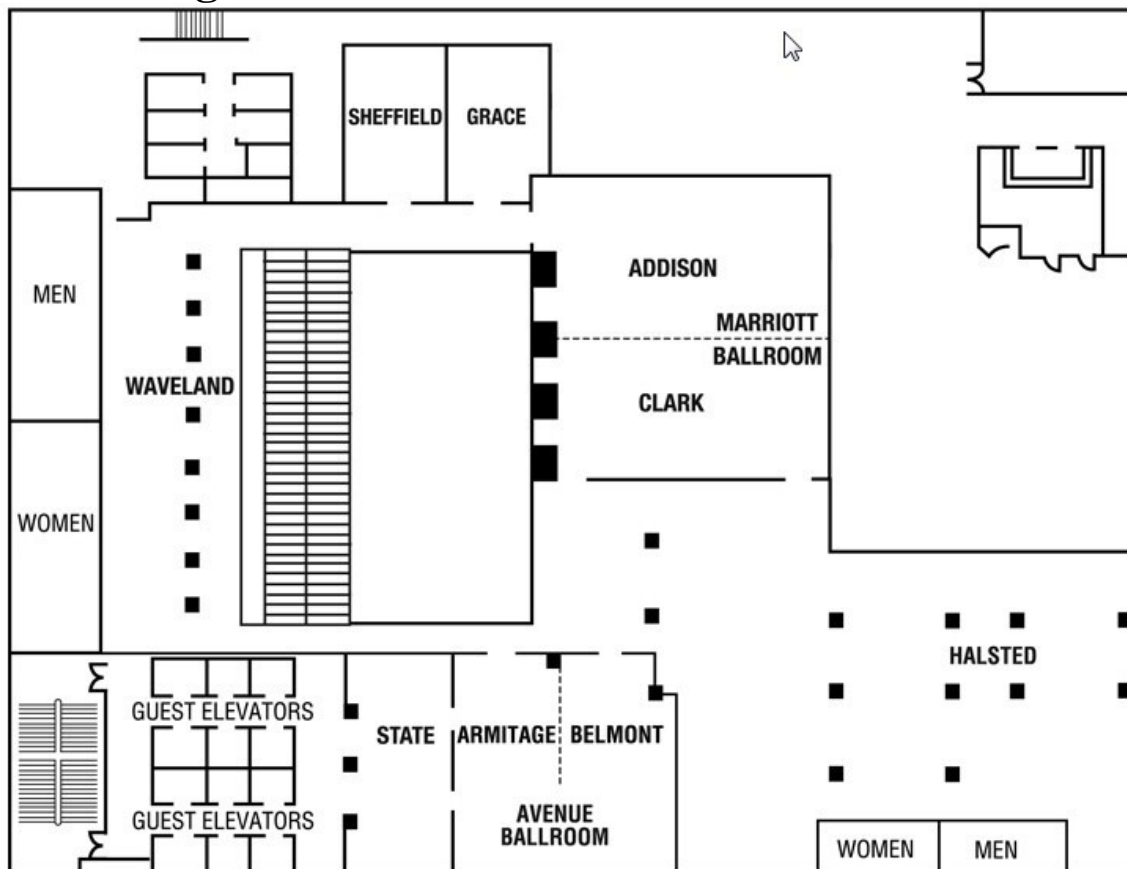
**Poster Presenters must be with their competition entry posters for possible judge interviews and must wear their name badge during their presentation.**

Poster Boards are 4 ft tall x 8 ft wide. Poster presenters must furnish their own tacks.

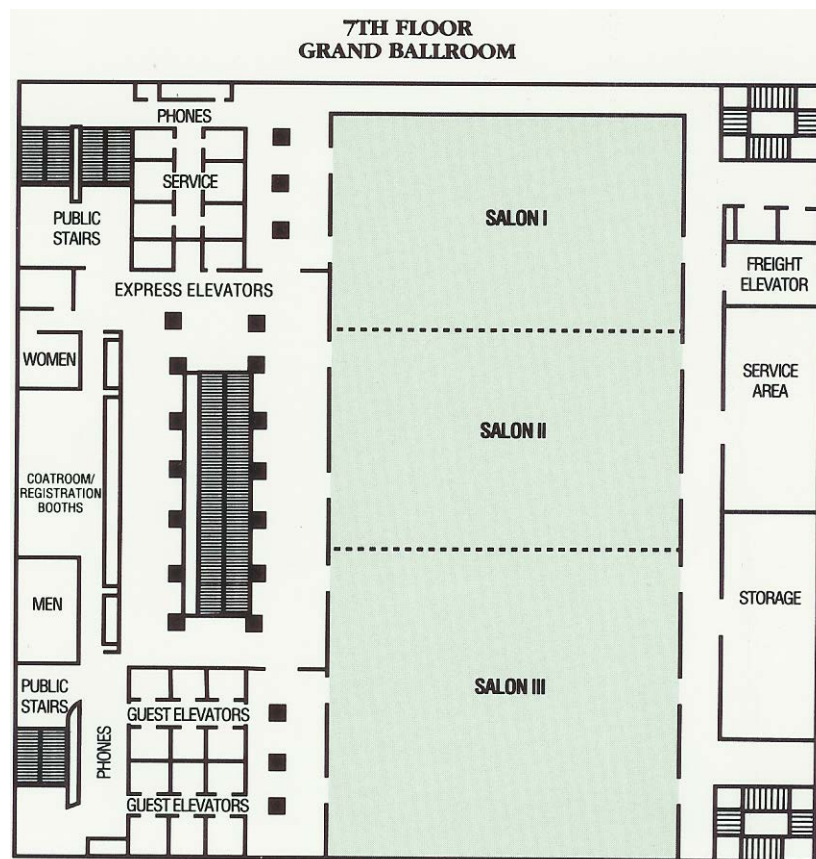
# Chicago Marriott, Floor Plan - 5<sup>th</sup> and 6<sup>th</sup> Floors



# Chicago Marriott Floor Plan – 4<sup>th</sup> and 7<sup>th</sup> Floors



Avenue Ballroom on 4<sup>th</sup> Floor



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Program and Proceedings compiled and edited by L. Susanne Squires, CRWAD Administrative Assistant.

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CRWAD

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## **CRWAD**

### **Meeting and Organization Information**

The Conference of Research Workers in Animal Diseases (CRWAD) was founded in Chicago in 1920. The CRWAD Annual Meeting is held on a Sunday, Monday and Tuesday of December, and consists of oral and poster presentations. The presentations are arranged into the following 10 Sections, according to the primary topic of the presentation: Bacterial Pathogenesis, Biosafety and Biosecurity, Companion Animal Epidemiology, Ecology and Management of Foodborne Agents, Epidemiology and Animal Health Economics, Immunology, Pathobiology of Enteric and Foodborne Pathogens, Respiratory Diseases, Vector-Borne and Parasitic Diseases, and Viral Pathogenesis. The oral presentations are limited to 15 minutes, with a recommendation of ten minutes presentation and five minutes for discussion. There are usually seven or eight Sections meeting simultaneously, so the time limit is judiciously recognized in order to allow attendees to move from Section to Section to listen and discuss the presentations of most interest to them. The two general Poster Sessions are held Sunday evening and Monday afternoon. Attendance is limited to members, nonmembers who are member applicants or who are presenters at the meeting, and invited guests. The attendance has ranged from 450 to 500 for the past several years, with attendees from countries throughout the world.

The PROCEEDINGS of the annual meeting are published each year. A limited number of PROCEEDINGS is available for the years prior to 1995 from the Executive Director. CRWAD distributes the Proceedings. Prospective members should be actively engaged in research or research administration. Meeting information and membership applications may be obtained by contacting the Executive Director or by visiting our web site.

ABSTRACTS ARE AVAILABLE AT THE ON-LINE MEETING PLANNER AND ITINERARY BUILDER.  
<http://www.cvmbs.colostate.edu/mip/crwad/>

### **Purpose Statement**

The Conference of Research Workers in Animal Diseases (CRWAD) was established in 1920. CRWAD is a non-profit organization and has been so since its origin. The sole purpose of CRWAD is to discuss and disseminate the most current research advances in animal diseases. Graduate students and industry and academic professionals present and discuss the most recent advances on subjects of interest to the CRWAD and of importance to the global livestock and companion animal industries. The oral and poster abstracts of new and unpublished data presented at the meeting sessions are published each year in the CRWAD Proceedings.

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**CRWAD Web Page Address: <http://www.cvmbs.colostate.edu/mip/crwad/>**

## 2014 Officers

President - David A. Benfield

Vice President - Roman R. Ganta

Council Members:

Laurel J. Gershwin (2010 – 2014); Paul S. Morley (2011 – 2015);

Christopher Chase (2012 - 2016); Qijing Zhang (2013 – 2017)

Executive Director - Robert P. Ellis

Administrative Assistant – L. Susanne (Suzy) Squires

### Recent Past Presidents

Rodney Moxley - 2013

Donald L. Reynolds – 2012

Eileen L. Thacker – 2010

Richard E. Isaacson - 2008

Prem Paul - 2006

Janet MacInnes - 2004

Franklin A. Ahrens - 2002

Leon N. D. Potgieter - 2000

Donald G. Simmons - 1998

Patricia E. Shewen - 1996

Ronald D. Schultz - 1994

Richard F. Ross - 1992

Lynette B. Corbeil - 1990

Laura L. Hungerford - 2011

Bill Stich - 2009

Lynn A. Joens - 2007

Ian Gardner - 2005

Katherine M. Kocan - 2003

Linda J. Saif - 2001

M. D. Salman - 1999

Bert E. Stromberg - 1997

Bradford B. Smith - 1995

Lawrence H. Arp - 1993

Robert M. Corwin - 1991

William C. Wagner - 1989

### The Dedicatee Tradition

Each year, we select a Life member who has made outstanding contributions to CRWAD and to animal disease research to be honored as the Dedicatee for the CRWAD Annual Meeting. This tradition was initiated in 1974. Each Dedicatee is invited to attend the Annual Meeting as our guest. At the Business Meeting, the meeting is formally dedicated to the Dedicatee and the Dedicatee is given a plaque and an honorarium. Past Dedicatees and the 2014 Dedicatee are listed below:

W. R. Hinshaw	1974
H. C. H. Kernkamp	1976
C. H. Brandley	1978
A. G. Karlson	1980
L. C. Ferguson	1982
Carl Olson, Jr.	1984
Ben S. Pomeroy	1986
Earl Splitter	1988
R. Allen Packer	1990
Alvin F. Weber	1992
Erwin M. Kohler	1994
Lyle E. Hanson	1996
J. Brian Derbyshire	1998
Leroy Coggins	2000
Johannes Storz	2002
Harley W. Moon	2004
Leland E. Carmichael	2006
Sidney A. Ewing	2008
Samuel K. Maheswaran	2010
William C. Wagner	2012
Donald C. Robertson	2014

S. H. McNutt	1975
R. W. Dougherty	1977
S. F. Scheidy	1979
I. A. Merchant	1981
Fred Maurer	1983
Charles Cunningham	1985
Norman Levine	1987
Marvin J. Twiehaus	1989
Donald A. Barnum	1991
E. O. Haelterman	1993
Edward H. Bohl	1995
Gordon R. Carter	1997
Bernard C. Easterday	1999
David P. Anderson	2001
Alexander J. Winter	2003
William L. Mengeling	2005
Richard F. Ross	2007
Norman F. Cheville	2009
Donald G. Simmons	2011
Fredric W. Scott	2013





## 2014 CRWAD Dedicatee – Donald C. Robertson

Dr. Don Robertson grew up on a 160 acre dairy farm outside of Rockford, Illinois. He started thinking about college at an early age since asthma and severe allergies prevented him from pursuing a career associated with agriculture. He attended the University of Dubuque, Dubuque, IA where he received a B.S in chemistry.

His first exposure to research on animal pathogens was in 1962 at the National Animal Disease Laboratory (NADL) in Ames, IA. His graduate research at the NADL focused on identification and characterization of the pathways and key enzymes that catabolize glucose in *Brucella abortus*, *Brucella suis*, and *Brucella melitensis*. After earning the PhD in biochemistry from Iowa State University in 1967, Don moved to the Department of Biochemistry at Michigan State University for a post-doctoral fellowship focused on purification and characterization of proteins. He joined the faculty in the Department of Microbiology at the University of Kansas on June 1, 1970 where he remained until he took a position as Head of Microbiology and Biochemistry at the University of Idaho in 1992. He accepted a position as Associate Director of Research and Extension in the Idaho Experiment Station in 1998 and moved to Kansas State University in 2000 as Associate Dean for Research and Graduate Studies and Professor of Microbiology in the College of Veterinary Medicine. In 2005 he returned to full-time research and teaching and retired in 2008 as Professor emeritus of Microbiology.

Dr. Robertson was blessed during his career with numerous outstanding graduate students and post-doctoral fellows who have gone on to successful careers in academia and industrial positions. Fifteen students earned their PhDs working in his laboratory. His initial research program at the University of Kansas focused on mechanisms used by *B. abortus* and other facultative intracellular parasites to survive within phagocytic cells and often grow at a rate similar to complex media. Sugar transport systems, the erythritol catabolic pathway, and lipopolysaccharides associated with smooth and rough strains of *B. abortus* were characterized. The association, ingestion, degranulation and killing reactions of polymorphonuclear leukocytes (PMNs) incubated with smooth and rough strains of *B. abortus* were characterized. In addition, the brucellacidal activity of PMN granule extracts against smooth virulent and rough avirulent strains was determined.

In 1975 his laboratory began studies on the pathogenesis of enterotoxigenic *Escherichia coli* (ETEC), which cause disease in neonatal animals (piglets, calves and lambs), human infants and travelers to underdeveloped countries. The primary focus was on two kinds of enterotoxins that cause watery diarrhea and are produced after adherence of ETEC to

small intestinal cells. One enterotoxin is a low-molecular heat-stable peptide containing 18 or 19 amino acids known as STa and the second is a high molecular protein enterotoxin consisting of two kinds of subunits known as the heat-labile enterotoxin (LT). Both enterotoxins were purified to homogeneity and characterized with respect to chemical and immunological properties and association with intestinal cells. Antisera raised to STa coupled to protein carriers were used to develop both radioimmunoassays (RIAs) and enzyme linked immunosorbent assays (ELISAs). Purified human and porcine LTs were used to raise specific antisera and characterize the immunological properties and cross reactions of LTs produced by human and porcine ETEC clinical isolates. Deletion mutants of the STa receptor, which is a transmembrane protein with several functional domains including the extracellular binding domain for STa, and the intracellular enzyme activity that is activated and converts intracellular guanosine triphosphate (GTP) to guanosine cyclic 3', 5'- phosphate(cGMP), were used to study the mechanism of action of STa. The heat-stable enterotoxin produced by *Yersinia enterocolitica* (YSTa) was purified to homogeneity and characterized with respect to chemical and immunological properties. Also, studies were performed to determine virulence factors associated with atypical strains of *Y. enterocolitica* isolated from patients with diarrheal disease that do not produce a classical heat-stable enterotoxin.

Don taught undergraduate courses in General Microbiology, Pathogenic Microbiology and a graduate level course in Mechanisms of Microbial Pathogenicity at the University of Kansas. He taught a graduate level course in Pathogenic Mechanisms at the University of Idaho. Also, he participated in a team-taught microbiology course for second year veterinary students in the College of Veterinary Medicine, KSU from 2005 to 2007.

Dr. Robertson served on the Bacteriology and Mycology 2 Study section, National Institutes of Health from 1978 to 1983 and the Cholera Panel, U.S. – Japan Cooperative Medical Science Program from 1984 to 1992. He was granted a life membership in the Conference of Research Workers in Animal Diseases in 2009. Also, he was a member of the editorial boards for Infection and Immunity and Applied and Environmental Microbiology from 1983 to 1996. He served as an administrative advisor for the Minor Use Animal Drug Program (NRSP-7) from 1999 to 2005. He is a member of several professional organizations including the American Society of Microbiology, American Academy of Microbiology, American Society of Biochemistry and Molecular Biology, American Association of University Professors, American Society for Advancement of Science, Sigma XI and Gamma Sigma Delta.

Don has been married to his wife Ronna for 52 years with 2 daughters and 2 grandchildren. His hobbies include working on classic Ford cars, serving as president of the local classic car club for the past two years, service work with the Manhattan Lions Club, reading, and working in his yard.

**2014 CRWAD - Keynote Speaker - Viral Pathogenesis Section and  
ACVM Distinguished Veterinary Microbiologist**

**Dr. Siba K. Samal**

Professor of Virology, Department of Veterinary Medicine , University of Maryland,  
College Park, MD

**Abstract No. 178 - Title:** Viral Pathogenesis: Lessons Learned from Newcastle Disease Virus.

**Tuesday**, December 9, 10:00 AM - Los Angeles/Miami/Scottsdale, 5<sup>th</sup> Floor

Dr. Samal received B.V.Sc. & A.H. in 1976 from Orissa Veterinary College, India, his M.V.Sc. from Indian Veterinary Research Institute in 1978, his M.S. and Ph.D. from Texas A&M University in 1981 and 1985, respectively. He received post-doctoral training at Baylor College of Medicine in 1985-86 and at Plum Island Animal Disease Center in 1986-88. Dr. Samal is a Diplomate of the American College of Veterinary Microbiologists and a Fellow of the American Academy of Microbiology.

He began his career as an Assistant Professor at the University of Maryland in 1988 and became a full professor in 1998. Dr. Samal was a visiting scientist at the NIH in 1993-94. He has been the chairperson of the Department of Veterinary Medicine and Associate Dean of the Virginia-Maryland Regional College of Veterinary Medicine since 2001. Over the years, he has worked on blue tongue virus, bovine respiratory syncytial virus, aquareovirus, Newcastle disease virus, and other avian paramyxoviruses. His current research is focused on development of avian paramyxovirus vectored vaccines for human and animal diseases. He is author or co-author of more than 160 refereed scientific publications, more than 120 published abstracts, and 13 book chapters. He edited a book entitled, "The Biology of Paramyxoviruses," and is currently the editor of four scientific journals. As Principal Investigator, Dr. Samal has obtained more than \$10 million in extramural research funding. He holds three U.S. patents. He has served as major advisor for 24 doctoral students, four M.S. students, and 18 post-doctoral fellows. Throughout his career, Dr. Samal has received numerous prestigious awards, including the 1984 John Paul Delaplane Award at Texas A&M, the 1990 Outstanding Invention Award at the University of Maryland, the 2000 Award for Research Excellence at the Virginia Tech University, and the 2007 Dean Gordon M. Cairns Award at the University of Maryland.

**2014 CRWAD - Keynote Speaker - Immunology Section  
AAVI Distinguished Veterinary Immunologist Award**

**Dr. Hyun Soon Lillehoj**, Animal Biosciences and Biotechnology, Beltsville Agricultural Research Center, U.S. Department of Agriculture, Agricultural Research Service

**Abstract No. 107 - Title:** Challenges with Poultry Immunology and Disease Research: Immune reagent development and maintaining genetic breeds of poultry

**Monday, December 8, 1:30 PM - Salons F/G/H, 5<sup>th</sup> Floor**

**Hyun Soon Lillehoj, Ph.D.**, received her B.S. degree in Biology from the University of Hartford, M.S. degree in Microbiology from the University of Connecticut, and Ph.D. in Immunology from Wayne State University, School of Medicine. After graduation, she was a NIH post-doctoral fellow in the Department of Immunology and Microbiology, Wayne State University to conduct research on the immunology of prostate cancer and immunogenetics of autoimmune diseases. In 1981, she was appointed as a staff fellow in the Laboratory of Immunology, NIAID, NIH where she studied T-cell immunity. Since 1984, Dr. Lillehoj worked at the Agricultural Research Service of the USDA at the Beltsville Agricultural Research Center. Since joining the USDA-ARS, she has progressively risen in the ranks to where she is now highest grade level, Supergrade (ST). Her research career has focused on the immunobiology of host-pathogen interactions, vaccine development, mucosal immunology, immunogenetics and development of antibiotic alternative strategies. Dr. Lillehoj developed the first set of mouse monoclonal antibodies detecting chicken lymphocyte subpopulations that have been commercialized and used by poultry scientists world-wide and have been instrumental for investigation of avian cell-mediated immunity. More recently, Dr. Lillehoj constructed the first chicken intestinal cDNA microarray which has been of seminal importance in national and international poultry genomics research and developed and commercialized many novel antibiotic alternative strategies. Her research has resulted in more than 390 papers in peer-reviewed journals, 18 book chapters, 600 meeting abstracts, and 14 patents. She has been awarded more than \$ 20 million in research funding, including 8 CSREES NRI, BARD, IFASA, and Food Safety Initiative grants, and 45 formal collaborations (CRADAs) with private industry since she joined ARS. In addition, she has served on numerous editorial boards, national grant panels, award and technical committees of the AAAPV and PSA, and chaired multiple sessions at national and international meetings. Dr. Lillehoj holds adjunct professorships at the University of Delaware, the University of Maryland, Mississippi State University and the University of Guelph and has guided the research of 95 junior scientists and graduate students from Asia, Europe, and South America. Her accomplishments have been recognized by the BARC Technology Transfer Award (1998), the ARS Technology Transfer Award (1999), the Federal Laboratory Consortium (FLC) Technology Transfer Award (1999), the Helen Cecil Leadership Award (2001), the AVMA Pharmacia/Upjohn Animal Health Achievement Award (2001), the Korean Poultry Science Association Distinguished Scientist Award (2001), the Beltsville Agricultural Research Center Senior Scientist of the Year Award (2003), the ARS Outstanding Scientist of the Year Award (2004), Merck Achievement Award (2006), the Levine P.P Award (AAAPV, 2006), the Pfizer Animal Health (Embrex) Fundamental Science Award (2007), Beltsville ARS Technology Transfer Award (2008), and Phibro Animal Health Award (2011). Dr. Lillehoj will be inducted into the ARS Hall of Fame in September 2014.

## 2014 Mark Gearhart Memorial Graduate Student Award

CRWAD Presentation No. 085

8:45 AM, Tuesday, December 9 – Salons A/B/C/D, 5<sup>th</sup> Floor

*Mannheimia haemolytica* in feedlot cattle: associations with antimicrobial use, resistance and health outcomes.

Noelle R. Noyes<sup>1</sup>, K.M. Benedict<sup>1</sup>, S.P. Gow<sup>2</sup>, C.W. Booker<sup>3</sup>, S.J. Hannon<sup>3</sup>, T.A. McAllister<sup>4</sup>, P.S. Morley<sup>1</sup>; <sup>1</sup>Clinical Sciences, Colorado State University, Fort Collins, CO, USA, <sup>2</sup>Laboratory for Foodborne Zoonoses, University of Saskatchewan, Saskatoon, SK, Canada, <sup>3</sup>Feedlot Health Management Services, Okotoks, AB, Canada, <sup>4</sup>Lethbridge Research Center, University of Lethbridge, Lethbridge, AB, Canada.

*Mannheimia haemolytica* is a significant etiological agent in bovine respiratory disease in cattle. Objectives of this study were to explore risk factors for isolation of susceptible and resistant *M. haemolytica* in a commercial feedlot setting, and to explore associations between isolation and health outcomes.

Cattle (n=5,498) from 4 feedlots located in Alberta, Canada were randomly enrolled and sampled at arrival and later in the feeding period. Samples were cultured for *M. haemolytica* and tested for resistance to 21 antimicrobials. Records of antimicrobial use (AMU) and health events were collected. Inferential analysis was conducted using multivariable GEE logistic regression. Parenteral AMU rates were low, and resistance prevalence was <2% for most drugs. Parenteral drug administration within 7 days of sampling was associated with decreased likelihood of *M. haemolytica* isolation (OR 0.2, 95%CI =0.02 - 1.2, P=0.006), while parenteral AMU in penmates of enrolled cattle increased this likelihood (OR 1.5, 95%CI 1.05 - 2.2, P=0.02). Parenteral AMU was not associated with isolation of single-drug resistant *M. haemolytica*, but greatly increased the odds of recovering *M. haemolytica* resistant to  $\geq 2$  drugs (OR 23.9, 95%CI 8.4 - 68.3, P<0.0001). Cattle from which *M. haemolytica* was cultured on arrival were more likely to be diagnosed with fever within 10 days of arrival compared to culture-negative cattle (OR 1.7, 95%CI 1.1 - 2.4, P=0.07).

Contagious spread may underlie colonization and transmission dynamics, as AMU in pen mates of enrolled cattle increased the risk of isolating both susceptible and multiply-resistant *M. haemolytica*. AMU did not appear to be the primary driver of resistance in *M. haemolytica*, and AMU protocols that target high-risk and clinically ill cattle are likely efficacious.

# PROGRAM



## CRWAD THANKS THE FOLLOWING 2014 SPONSORS

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December 7-9

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## **CRWAD THANKS THE FOLLOWING 2014 SPONSORS**

Chicago Marriott, Downtown Magnificent Mile, Chicago, Illinois  
December 7-9

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**Bronze Medal Contributor \$1,000.00 and <\$2,500.00**

**MIDWEST VETERINARY SERVICES, CENTRAL STATES RESEARCH CENTRE**



The CRWAD Conference is supported by the National Institute for Food and Agriculture (NIFA) of the USDA Agriculture and Food Research Initiative (AFRI) two programs: AFRI Food Safety and AFRI Animal Health and Disease.

<http://www.cvmbs.colostate.edu/mip/crwad/>





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- In – vivo rat and gerbil models for efficacy evaluation of acaricides and nematocides
- Production of pathogen specific polyclonal serum

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- Autogenous Vaccine, RNA – the first non-living, recombinant vaccine for Rotavirus

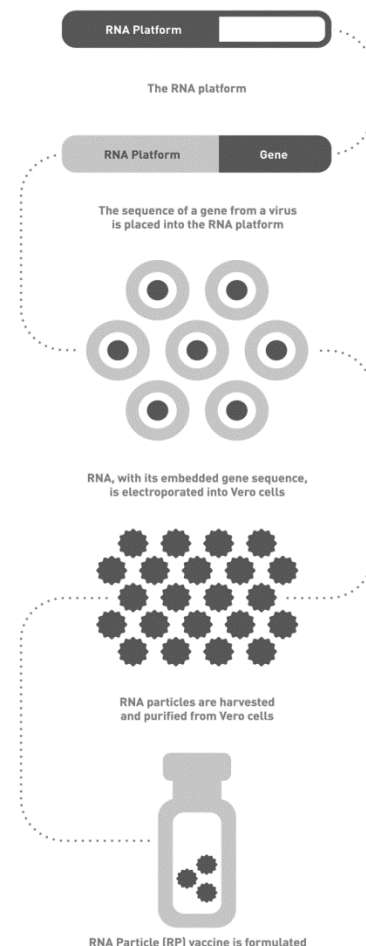
Our Production Platform technology, derived from an Alphavirus vector system, is the backbone of our Research and Development for products to safeguard U.S. agriculture from Foreign Animal Diseases including Foot and Mouth Disease, Classical Swine Fever, and emerging Coronaviruses.

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\*A USDA Conditional License is granted to products with a reasonable expectation of efficacy and that meet all safety and purity requirements. Harrisvaccines is currently pursuing a full USDA license for Porcine Epidemic Diarrhea Vaccine, RNA.

## VACCINE COMPARISON

	Traditional Vaccines			RNA Platform Vaccines
	Killed Virus	Modified Live	Extract	Particle (RP)
Antibodies	+	+	+	+
Cellular Immunity	-	+	-	+
May Cause Disease	-	+	-	-
Must Grow Agent	+	+	+	-
Adjuvant Required	+	-	+	-
Rapid Response	-	-	-	+



<http://www.cvmbs.colostate.edu/mip/crwad/sponsorship.htm>

**www.merial.com/**

DEDICATED TO ENHANCING  
the health and  
well-being of animals



**<http://www.cvmbs.colostate.edu/mip/crwad/sponsorship.htm>**

## CRWAD 2014 Exhibitors

### **Animal Health Research Reviews (AHRR)**

Animal Health Research Reviews provides an international forum for the publication of reviews and commentaries on all aspects of animal health. Papers include in-depth analyses and broader overviews of all facets of health and science in both domestic and wild animals. Major subject areas include physiology and pharmacology, parasitology, bacteriology, food and environmental safety, epidemiology and virology.

**<http://journals.cambridge.org/action/displayJournal?jid=AHR>**

### **Elsevier**

Elsevier is a world-leading, multi-media publisher of superior STM information products and services. Visit the Elsevier table in the exhibit area to browse our extensive selection of journals in veterinary science and related areas, pick-up free sample copies of selected journal titles and ask any questions that you may have!

**[www.elsevier.com/anivet](http://www.elsevier.com/anivet)**

### **GeneReach**

POCKIT Nucleic Acid Analyzer is a powerful point-of-need PCR detection tool that combines advanced insulated isothermal polymerase chain reaction (iiPCR) technology with user-friendly interface, and can offer clinical diagnostic laboratory, veterinarian and breeding industry an effective solution for disease surveillance.

**[www.genereach-us.com](http://www.genereach-us.com)**

### **List Biological Laboratories**

List Biological Laboratories produces highly purified bacterial toxins from infectious diseases: anthrax, pertussis, *Pasteurella*, *C. difficile*, *Staphylococcus*, Shiga, tetanus, botulinum, and lipopolysaccharides. List manufactures all major *Bordetella* virulence factors. List's experience includes: assay development, bacterial fermentation, protein purification. Contract manufacturing available for reagent or cGMP compliant proteins, adjuvants, and biotherapeutics.

**<http://www.listlabs.com/>**

### **Mabtech, Inc.**

Mabtech is a leader in the development of ELISpot products, technology and methods for detection of T and B-cell responses. Newer developments include FluoroSpot for detecting dual secreting cells. Other products include ELISA kits for detection of cytokines, immunoglobulins and apolipoproteins. Mabtech products are for Research Use Only.

**[www.mabtech.com](http://www.mabtech.com)**

## **CRWAD 2014 Exhibitors**

### **PerkinElmer**

Biological complexity raises questions requiring translational research from the well, to the cell, to the animal and back again. PerkinElmer enables you to approach your target from multiple perspectives: locate, detect and quantitate your biology of interest; analyze and understand it in wider physiological contexts.

**[www.perkinelmer.com](http://www.perkinelmer.com)**

### **Qiagen**

Qiagen is a provider of sample and assay technologies for molecular diagnostics, applied testing, academic and pharmaceutical research. Consolidated under the Dutch holding Qiagen N.V., the company operates more than 35 offices in over 20 countries.

**<http://www.qiagen.com/>**

### **Tetracore, Inc.**

Tetracore is an industry leader in the development of rapid tests for agricultural animal diseases. Tetracore's dried qPCR tests are ideal for surveillance monitoring and the EZ-PRRSV MPX 4.0 reagents are the industry gold standard for high throughput PRRSV detection. The T-COR 4 instrument allows for qPCR testing in the field.

**[www.tetracore.com](http://www.tetracore.com)**

## **2014 CRWAD Keynote Speakers and Titles**

### **Bacterial Pathogenesis Section – Dr. Richard E. Isaacson**

Department of Veterinary & Biomedical Science, University of Minnesota, St. Paul, MN  
Monday, December 8, 10:45 AM - Avenue Ballroom, 4<sup>th</sup> Floor

**No. 009 - Title** – Towards an understanding of Salmonella persistence in pigs.

### **Biosafety and Biosecurity Section – Dr. Helen Aceto**

Associate Professor of Epidemiology, Director of Biosecurity, New Bolton Center,  
Department of Clinical Studies, University of Pennsylvania School of Veterinary Medicine,  
Kennett Square, PA

Monday, December 8, 3:00 PM - Denver/Houston Room, 5<sup>th</sup> Floor

**No. 026 - Title** - Biosecurity, a biography: ten years of hard lessons.

### **Companion Animal Epidemiology, Ecology & Management of Foodborne Agents, and Epidemiology & Animal Health Economics Sections – Dr. Brian McCluskey**

Chief Epidemiologist at USDA-APHIS-Veterinary Service, Fort Collins, CO

Tuesday, December 9, 8:00 AM - Salons A/B/C/D, 5<sup>th</sup> Floor

**No. 084 - Title** - The Symbiology of Epidemiologic Pursuits of Academia and Government.

### **Immunology Section – Distinguished Veterinary Immunologist – Dr. Hyun Soon Lillehoj**

Animal Biosciences & Biotechnology Laboratory, USDA-ARS, Beltsville, MD

Monday, December 8, 1:30 PM - Salons F/G/H, 5<sup>th</sup> Floor

**No. 107 - Title** - Challenges with Poultry Immunology and Disease Research: Immune reagent development and maintaining genetic breeds of poultry.

### **Pathobiology of Enteric and Foodborne Pathogens – Dr. Timothy J. Johnson**

Department of Veterinary & Biomedical Science, University of Minnesota, St. Paul, MN

Monday, December 8, 8:45 AM – Los Angeles/Miami Room, 5<sup>th</sup> Floor

**No. 128 - Title** – Plasmids of enteric bacterial pathogens: past, present, and future challenges.

### **Respiratory Diseases - Dr. David W. Horohov**

Gluck Equine Research Center, University of Kentucky, Lexington, KY

Monday, December 8, 3:45 PM - Indiana/Iowa Room, 6<sup>th</sup> Floor

**No. 152 - Title** - Regulation of interferon-gamma gene expression in foals and its relationship to susceptibility to Rhodococcus equi.

### **Vector-Borne and Parasitic Diseases – Dr. Edward Breitschwerdt**

Department of Clinical Sciences and the Center for Comparative Medicine and Translational Research,  
College of Veterinary Medicine, North Carolina State University, Raleigh, NC

Monday, December 8, 10:00 AM - Denver/Houston Room, 5<sup>th</sup> Floor

**No. 159 - Title** - Bartonellosis: A One Health Approach to an Emerging Infectious Disease.

### **Viral Pathogenesis Section – Dr. Siba K. Samal – Distinguished Veterinary Microbiologist**

Department of Veterinary Medicine, University of Maryland, College Park, MD

Tuesday, December 9, 10:00 AM - Los Angeles/Miami/Scottsdale, 5<sup>th</sup> Floor

**No. 178 - Title** – Viral Pathogenesis: Lessons Learned from Newcastle Disease Virus.

## 2014 CRWAD - Keynote Speaker - Bacterial Pathogenesis Section

### Dr. Richard E. Isaacson

Department of Veterinary & Biomedical Science, University of Minnesota, St. Paul, MN

**Abstract No. 009 - Title:** Towards an understanding of Salmonella persistence in pigs.

**Monday,** December 8, 10:45 AM - Avenue Ballroom, 4<sup>th</sup> Floor

**Richard Isaacson, Ph.D.,** Professor of Microbiology, Department of Veterinary and Biomedical Sciences, University of Minnesota, St. Paul, MN

Richard Isaacson received his Ph.D. from the Department of Microbiology, University of Illinois under the mentorship of Jordan Konisky. He then spent four years working with Harley Moon at the National Animal Disease Center, Ames, IA. He has had faculty positions at the University of Michigan, Department of Epidemiology, the University of Illinois, Department of Veterinary Pathobiology, and the University of Minnesota, Department of Veterinary and Biomedical Sciences. He also served as Manager of Immunology and Infectious Diseases, Pfizer Central Research, Groton, CT. While at the University of Minnesota, he served as department chair.

Dr. Isaacson was elected a fellow in the American Academy of Microbiology, is the chair of division Z (Animal Microbiology) American Society for Microbiology, serves on two journal editorial boards and is associate editor for the journal Zoonoses and Public Health.

My laboratory is studying the molecular basis of pathogenesis of two pathogens: *Escherichia coli* and *Salmonella enterica* serovar Typhimurium. A common theme in each project is the identification of unique genes required for pathogenesis, understanding the functions of those genes, and determining the mechanisms controlling their expression. Our studies on *S. enterica* serovar Typhimurium are directed toward an understanding of the mechanism whereby *S. Typhimurium* can persistently infect pigs, yet not cause disease. We have isolated two phenotypic variants one of which can attach to porcine villous epithelial cells and the other cannot. Using RNASeq we identified 85 genes that were up regulated in the adhesive phenotype. We also identified a gene, *lrhA*, which increases the rate of phase variation from the non-adhesive to the adhesive phenotype. Our work on *E. coli* has focused on attachment of enterotoxigenic strains to mucosal surfaces. More recently our work has been on nosocomial septicemia using in vivo gene expression technology to identify genes exclusively expressed during disease. In the collection of identified genes are 9 unique genes that are mainly present in other pathogenic strains that cause urinary tract infections and sepsis. Mutations in each of these genes resulted in attenuation of the *E. coli* strain in vivo. One gene has been characterized as part of a novel iron transport system, term Fit. In a third project, we are studying microbial population shifts in the intestines of pigs in response to treatment with an antimicrobial growth promoter to determine if there are shifts microbiome of treated pigs. A major conclusion from this work is that tylosin may accelerate the maturation of the adult gut microbiome and this facilitates growth promotion. We also are assessing the microbiota in the lungs of patients who have had lung transplants and those that have chronic pulmonary obstructive disease (COPD). Both patient groups had different lung microbiomes and each had greater diversity of microbes compared to healthy patients.

## 2014 CRWAD - Keynote Speaker - Biosafety and Biosecurity Section

### Dr. Helen Aceto

Associate Professor of Epidemiology, Director of Biosecurity, New Bolton Center,  
Department of Clinical Studies, University of Pennsylvania School of Veterinary Medicine,  
Kennett Square, PA

**Abstract No. 026 - Title:** Biosecurity, a biography: ten years of hard lessons  
**Monday, December 8, 3:00 PM - Denver/Houston Room, 5<sup>th</sup> Floor**

Helen Aceto PhD VMD

Primary Research Interests:

Epidemiology of *Salmonella* in cattle and horses, development and implementation of biosecurity and disease control strategies for animal healthcare and community settings, epidemiology of equine colic.

Other Research Interests:

Assessment of veterinary surveillance models for hospital and community-based applications. Ecology of antimicrobial resistant pathogens in animal health and agricultural settings, antimicrobial use patterns in livestock and companion animals. Equine performance assessment.

Brief Career Synopsis:

Helen Aceto graduated from the University of Dundee, School of Medicine in Scotland in 1977 with a B.Sc. honors degree in Pharmacology. She then spent almost ten years in industry-based research with Imperial Chemical Industries (ICI) pharmaceuticals division (now Astra-Zeneca) in Cheshire, England. In 1986, she moved to the Department of Pharmacology at Temple University School of Medicine in Philadelphia, USA. She received a Ph.D. in Pharmacology from Temple in 1993. In 1997 she received a V.M.D. from the University of Pennsylvania School of Veterinary Medicine. She remained at Penn Vet after graduation spending 6 years as first, a research associate in animal health economics, and then a lecturer in epidemiology. During that period she was also a member of the Pennsylvania Animal Diagnostic Laboratory System's Field Investigation Group based at New Bolton Center. She has been the Director of Biosecurity at the George D. Widener Hospital for Large Animals for the last 10 years and a faculty member in Epidemiology since 2005. The Widener hospital has about 6,000-6,500 patient visits annually, including approximately 1,200 emergency admissions, and as such sees a large number of animals with infections that are zoonotic in nature and/or nosocomial threats. Dr. Aceto's clinical expertise lies in two related areas, zoonotic diseases in large animals, and biosecurity and infection control. In particular, she has extensive experience in *Salmonella* epidemiology, diagnostics, and control.



**2014 CRWAD - Keynote Speaker for the Companion Animal Epidemiology Section,  
Ecology and Management of Foodborne Agents Section, and Epidemiology and  
Animal Economics Section**

**Dr. Brian McCluskey**

Chief Epidemiologist at USDA-APHIS-Veterinary Service, Fort Collins, CO

**Abstract No. 084 - Title:** The Symbiology of Epidemiologic Pursuits of Academia and Government.

**Tuesday,** December 9, 8:00 AM - Salons A/B/C/D, 5<sup>th</sup> Floor

**Brian McCluskey** received a Doctorate in Veterinary Medicine from Washington State University in 1987 and following graduation was in large animal practice, primarily dairy, in western Washington state. He joined APHIS in 1990 and was stationed in Charleston, West Virginia as a section veterinary medical officer. He was then sponsored by APHIS Cattle Diseases Staff in a graduate program at the University of Florida and after receiving his Masters degree in epidemiology, was stationed at the Colorado Area Office as the Area Epidemiology Officer. During this time he became a Diplomate in the American College of Veterinary Preventive Medicine. Brian then moved to the Center for Animal Health Monitoring in Fort Collins, CO as the Dairy Commodity Support Analyst. He received a PhD in Epidemiology at Colorado State University in 2003 and from 2003 to 2007 was the Director of the USDA's National Surveillance Unit. He joined the Senior Executive Service in 2007 as the Director of Veterinary Services Western Region. Brian was named APHIS, Veterinary Services Chief Epidemiologist, a senior scientist level position, in 2011.

**2014 CRWAD - Keynote Speaker**  
**Pathobiology of Enteric and Foodborne Pathogens Section**

**Dr. Timothy J. Johnson**

Department of Veterinary & Biomedical Science, University of Minnesota St. Paul, MN

**Abstract No. 128 - Title:** Plasmids of enteric bacterial pathogens: past, present, and future challenges.

**Monday**, December 8, 8:45 AM – Los Angeles/Miami Room, 5<sup>th</sup> Floor

Dr. Timothy Johnson received his BS in Microbiology (2000) and PhD in Molecular Pathogenesis (2004) from North Dakota State University in Fargo, ND. He went on to study *Escherichia coli* pathogenomics and plasmid biology during his postdoctoral fellowship at Iowa State University. In his current position as Associate Professor of Microbiology at the University of Minnesota, Dr. Johnson continues to focus on the biology of plasmids of enteric bacteria, while also studying bacterial communities and pathogens of the poultry gastrointestinal tract.

## 2014 CRWAD - Keynote Speaker - Respiratory Diseases Section

### **Dr. David W. Horohov**

Gluck Equine Research Center, Department of Veterinary Science, University of Kentucky  
Lexington, KY

**Abstract No. 152 - Title:** Regulation of interferon-gamma gene expression in foals and its relationship to susceptibility to *Rhodococcus equi*.

**Monday**, December 8, 3:45 PM - Indiana/Iowa Room, 6<sup>th</sup> Floor

### **Dr. David W. Horohov:**

Jes E. and Clementine M. Schlaikjer Endowed Chair , Department of Veterinary Science,  
Maxwell H. Gluck Equine Research Center, University of Kentucky, Lexington, KY

B.S. 1978 Pennsylvania State University

M.S. 1981 Purdue University

Ph.D. 1985 University of Tennessee

Staff Fellow, 1986-1988, FDA, Bethesda, MD

Professor of Veterinary Immunology, 1988-2003, Louisiana State University, Baton Rouge, LA

My research program focuses on the immune response of horses to various infectious and non-infectious diseases. The emphasis of this work has been on the identification and characterization of equine cytokines and the role they play in protective and pathologic immune responses. My research group has developed assays to detect these cytokines in various biological samples and used these assays have been used to better characterize the protective immune responses of horses to bacterial, viral and parasitic infections, *as well as the pathologic response in autoimmune and allergic diseases. The current research focus of my laboratory is on the characterization of the immune response* of foals and its relationship to their unique susceptibility to *Rhodococcus equi*, an important cause of bronchopneumonia. We have recently shown that foals less than 3 weeks of age are highly susceptible to infection with *R. equi* and that this susceptibility coincides with reduced expression of interferon-gamma. We are interested in better understanding the mechanism responsible for this susceptibility to *R. equi* in order to develop more effective prevention strategies for this disease.

## **2014 CRWAD - Keynote Speaker - Vector-Borne and Parasitic Diseases Section**

**Dr. Ed Breitschwerdt**, Department of Clinical Sciences and the Center for Comparative Medicine and Translational Research , College of Veterinary Medicine, North Carolina State University, Raleigh, North Carolina, USA

**Abstract No. 159 - Title:** Bartonellosis: A One Health Approach to an Emerging Infectious Disease.

**Monday**, December 9, 10:00 AM - Denver/Houston Room, 5<sup>th</sup> Floor

Edward B. Breitschwerdt, BS, DVM

University attended: University of Georgia; Year graduated 1974

Type of practice or specialty: (diplomate) Internal Medicine (Companion Animal)

**Dr. Edward B. Breitschwerdt** is a professor of medicine and infectious diseases at North Carolina State University College of Veterinary Medicine. He is also an adjunct professor of medicine at Duke University Medical Center, and a Diplomate, American College of Veterinary Internal Medicine (ACVIM). Dr. Breitschwerdt directs the Intracellular Pathogens Research Laboratory in the Center for Comparative Medicine and Translational Research at North Carolina State University. He also co-directs the Vector Borne Diseases Diagnostic Laboratory and is the director of the NCSU-CVM Biosafety Level 3 Laboratory.

A graduate of the University of Georgia, Breitschwerdt completed an internship and residency in Internal Medicine at the University of Missouri between 1974 and 1977. He has served as president of the Specialty of Internal Medicine and as chairman of the ACVIM Board of Regents. He is a former associate editor for the *Journal of Veterinary Internal Medicine* and was a founding member of the ACVIM Foundation.

Breitschwerdt's clinical interests include infectious diseases, immunology, and nephrology. For over 20 years, his research has emphasized vector-transmitted, intracellular pathogens. Most recently, he has contributed to cutting-edge research in the areas of animal and human bartonellosis. In addition to authoring numerous book chapters and proceedings, Dr. Breitschwerdt's research group has published more than 300 manuscripts in peer-reviewed scientific journals. In 2012, he received the North Carolina State University Alumni Association Outstanding Research Award and in 2013 he received the Holladay Medal, the highest award bestowed on a faculty member at North Carolina State University.

## **2014 CRWAD AND SATELLITE MEETINGS (Alphabetically listed)**

ABSTRACTS AVAILABLE AT THE ON-LINE MEETING PLANNER AND ITINERARY BUILDER  
<http://www.cvmbs.colostate.edu/mip/crwad/>

### **CRWAD Registration – 5<sup>th</sup> Floor Foyer Registration Booth**

Sunday, Dec. 7, 10 AM - 5:30 PM  
Monday, Dec. 8, 7:00 AM - Noon, 2 - 5 PM  
Tuesday, Dec. 9, 8 - 11 AM

### **CRWAD Researchers Reception and Poster Session I - Grand Ballroom Salon III - 7<sup>th</sup> Floor**

(Poster I Sections listed inside front cover)  
Sunday, Dec. 7, 6-8 PM - Reception  
Poster Session I Set-up - 4 PM Sunday (Section Posters are listed in the Summary Table)  
Remove Sunday session posters by 10:00 AM Monday  
First Poster Session - 6:30-8 PM, Sunday  
**All Attendees are Welcome. Please join us. Casual wear recommended.**

### **CRWAD Poster Session II - Grand Ballroom Salon III - 7<sup>th</sup> Floor**

Monday, Dec. 8 - 5:00 PM - 6:30 PM  
Poster Session II Set-up - 12:00 PM, Monday (Section Posters are listed inside the front cover)  
Remove posters immediately upon completion of Poster Session II, 6:30 PM.

### **CRWAD Students and Post-Docs Reception**

Sunday, Dec. 7, 5:00 PM – 5:45 PM, Los Angeles/Miami/Scottsdale Room, 5<sup>th</sup> Floor  
Name badge required  
Who should attend? Full Time Students, Post Docs, Council Members, Dedicatee, Keynotes, and other invited guests

### **American Association of Veterinary Immunologists (AAVI)**

Sunday, Dec. 7, **Board Meeting**  
1 PM - 5 PM – Indiana/Iowa Room - 5<sup>th</sup> Floor  
For more information contact Glenn Zhang [glenn.zhang@okstate.edu](mailto:glenn.zhang@okstate.edu) (AAVI Treasurer)  
or Laura Miller [laura.miller@ars.usda.gov](mailto:laura.miller@ars.usda.gov) (AAVI Vice-President).

### **AAVI Mini-Symposium (American Association of Veterinary Immunologists)**

Tuesday, Dec. 9, presented within the CRWAD Immunology Section, Salons F/G/H – 5<sup>th</sup> Floor  
Title: “The Analysis and the Role of the Microbiome in Immunology” - Interventions during early life impact the microbial colonization and immune development in livestock species.

### **American College of Veterinary Microbiologists (ACVM)**

Examination - Denver/Houston Room - 5<sup>th</sup> Floor  
Friday, Dec. 5, 12 PM - 8 PM (for 2014)  
Saturday, Dec. 6, 8 AM - 9 PM

Examination - Kansas City Room – 5<sup>th</sup> Floor  
Saturday, Dec. 6, 8 AM – 1 PM

Sunday, Dec. 7, Indiana/Iowa Room - 5<sup>th</sup> Floor  
8 AM - 9 AM - Examination Committee Meeting  
9 AM - 12 PM - Board of Governors  
Meeting. Attendance is by invitation only.  
For more information contact Josh Daniels.

### ACVM-CE Symposium – 4<sup>th</sup> Floor

Monday, Dec. 8, Avenue Ballroom  
(Presented within the CRWAD Bacterial Pathogenesis Section)

## **2014 CRWAD AND SATELLITE MEETINGS (Alphabetically listed)**

### **Animal Health Research Reviews (AHRR) Board Meeting**

Tuesday, Dec. 9, 7:00 - 8:30 AM – Grace Room - 4<sup>th</sup> Floor  
Section Editors and Editorial Board joint meeting.  
For more information contact Bill Stich, Editor in Chief

### **AVEPM – A workshop on the Basic reproductive ratio ( $R_0$ ) (open attendance)**

(Association for Veterinary Epidemiology and Preventive Medicine)  
Sunday, Dec. 7, 8:00 AM – 11:00 AM, Chicago Ballroom Salon E/F/G/H Room - 5<sup>th</sup> Floor  
For more information contact Annette O'Connor, Julie Funk or H. Morgan Scott.

### **ACVM-CM Mini-Symposium**

Monday, Dec. 8, included within the CRWAD Bacterial Pathogenesis Section, Avenue Ballroom, 4<sup>th</sup> Floor

### **AVEPM Schwabe Symposium - “Diseases, Dilemmas, Decisions: Epidemiological Tools to Find Answers for Difficult Disease Control Problems”**

A Symposium Honoring the Legacy of Dr. Roger Morris, Emeritus Professor of Animal Health, Massey University, New Zealand.

(Association for Veterinary Epidemiology and Preventive Medicine)  
Sunday, Dec. 7, 12:30 PM - 5 PM, Chicago Ballroom Salon E/F/G/H Room - 5<sup>th</sup> Floor

Formal presentation to Dr. Roger Morris will be during CRWAD Business Meeting, Tuesday, Dec. 9  
11:45 AM - 12:30 PM, Chicago Ballroom A/B/C/D, 5<sup>th</sup> Floor  
For more information contact Annette O'Connor, H. Morgan Scott, or Julie Funk.

### **AVEPM Business Meeting – Members only**

(Association for Veterinary Epidemiology and Preventive Medicine)  
Monday, Dec. 8, 11:30 AM – 1:30 PM – Salon A/B/C/D (Epi Section) Room - 5<sup>th</sup> Floor  
For more information contact Morgan Scott

### **CRWAD Council Meeting**

Saturday, Dec. 6, 5:30 PM - 9 PM - Great America Room - 6<sup>th</sup> Floor

### **CRWAD Business Meeting**

Tuesday, Dec. 9, 11:45 AM - 12:30 PM - Chicago Ballroom A/B/C/D - 5<sup>th</sup> Floor  
Dedication of the Meeting, Introduction of New Members, and Graduate Student Competition Awards  
New member applicants and all students entered in the competition are invited and encouraged to attend.

### **CRWAD Sponsorship Committee Meeting (report to the Council Meeting)**

Saturday, Dec. 6, 5:30 – 6:00 PM, Great America Room - 6<sup>th</sup> Floor

### **Distinguished Veterinary Immunologist Lecture by Dr. Hyun Soon Lillehoj**

Animal Biosciences & Biotechnology Laboratory, USDA-ARS, Beltsville, MD  
Monday, Dec. 8, 1:30 PM - Salons F/G/H, 5<sup>th</sup> Floor  
**Title** – Challenges with Poultry Immunology and Disease Research: Immune reagent development and maintaining genetic breeds of poultry.

### **Distinguished Veterinary Microbiologist is Dr. Siba K. Samal**

Professor of Virology, Department of Veterinary Medicine, University of Maryland, College Park, MD  
Tuesday, December 8, 10:00 AM - Los Angeles/Miami/Scottsdale, 5<sup>th</sup> Floor  
**Title** – Viral Pathogenesis: Lessons Learned from Newcastle Disease Virus.

**2014 CRWAD AND SATELLITE MEETINGS  
(Alphabetically listed)**

**Exhibitors - (Table Top)**      Sunday - Monday, Dec. 7-8, 5<sup>th</sup> Floor Foyer  
Sunday – 10 AM – 5:30 PM  
Monday - 7:45 AM – 5 PM (close Monday, Dec. 8, 5 PM)

AHRR  
Elsevier  
GeneReach  
List Biological Laboratories  
MabTech, Inc.  
PerkinElmer  
Qiagen  
Tetracore, Inc.

**Integrated Special Emphasis Project**

Minimizing Antibiotic Resistance Transmission throughout the Food Chain  
Saturday, Dec. 6  
11:00 AM - 5:00 PM, Northwestern/Ohio Room – 6<sup>th</sup> Floor

For more information contact H. Morgan Scott, Texas A&M University: 979-847-6197

**NC-1202 Enteric Diseases of Food Animals: Enhanced Prevention, Control and Food Safety**

Saturday, Dec. 6, 8 AM - 5 PM – Miami Room - 5<sup>th</sup> Floor  
Sunday, Dec. 7, 8 AM - 12 PM - Miami Room - 5<sup>th</sup> Floor  
Attendance is by invitation only.

For more information contact Gireesh Rajashekara email: [rajashekara.2@osu.edu](mailto:rajashekara.2@osu.edu)

**NC-229: Detection and Control of Porcine Reproductive and Respiratory Syndrome Virus and Emerging Viral Diseases of Swine**

Sunday, Dec. 7, 1 PM - 5PM, Denver/Houston/Kansas City Room

Attendance is open. For more information contact Dr. Fernando Osorio [fosorio1@unl.edu](mailto:fosorio1@unl.edu) or  
Kyoung-Jin Yoon [kyoon@mail.iastate.edu](mailto:kyoon@mail.iastate.edu)

**ABSTRACTS AVAILABLE AT THE ON-LINE MEETING PLANNER AND ITINERARY BUILDER**

**<http://www.cvmbs.colostate.edu/mip/crwad/>**

**The whole is not only more than but very different than the sum of its parts  
– A Symposium Honoring the Professional Legacy of Dr. Roger Morris –**



The Association of Veterinary Epidemiology and Preventive Medicine (AVEPM) is pleased to announce the program for the 2014 Schwabe Symposium honoring the professional achievements of Dr. Roger Morris. The symposium will be held in Chicago on **Sunday, Dec 7, 2014**, at the [Chicago Marriott, Downtown Magnificent Mile](#), Chicago, Illinois, just prior to the opening of the Conference of Research Workers in Animal Diseases. There is no registration fee for the symposium, and all are welcome to attend.

**"Diseases, Dilemmas, Decisions: Epidemiological Tools to Find Answers for Difficult Disease Control Problems"**

11:30 am	Light buffet lunch for attendees
12:25 pm	Introductory Remarks - Annette O'Connor
12:30 pm	<i>Spatial and temporal data analysis in support of decision making for complex animal health problems</i> – Dirk Pfeiffer (Royal Veterinary College, London UK)
1:05 pm	<i>Dealing with deficient and missing data</i> – Ian Dohoo (University of Prince Edward Island, Canada)
1:40 pm	<i>The value of information: Current challenges in surveillance implementation</i> – Katharina Staerk (Safoso, Switzerland)
2:15 pm	Break and Refreshments
2:55 pm	<i>Assessing disease incursion risk and response strategies in difficult environments</i> - Shiyong Wang (World Bank, Washington)
3:30 pm	<i>The dilemmas of a current emerging disease – porcine epidemic diarrhea in North America</i> – Peter Davies (University of Minnesota)
4:05 pm	Keynote address: <i>How epidemiological tools and insights can be used to produce decisions out of dilemmas for difficult disease control problems</i> – Dr. Roger Morris, Emeritus Professor of Animal Health, Massey University, New Zealand
4:50 pm	Closing comments
6:00 – 8:00 pm	CRWAD Researchers Reception and Poster Session I, Viewing

The Calvin W. Schwabe Award is presented annually by the AVEPM to honor lifetime achievement in veterinary epidemiology and preventive medicine. Previous recipients include Drs. Calvin Schwabe, Robert Anderson, James Steele, S. Wayne Martin, Clive Gay, David W. Hird, Hollis Erb, Preben Willeberg, Dale Hancock, Ian Dohoo and Yrjö Gröhn.



## **2014 Calvin W. Schwabe Award**

The 2014 Calvin W. Schwabe honoree is: **Dr. Roger Morris**, Emeritus Professor of Animal Health, Massey University, New Zealand

During the more than 40 years of his career, Professor Morris has established himself as one of the world's leading veterinary epidemiologists with an outstanding global profile as a researcher, educator and policy advisor. He has had a major impact on the veterinary profession and society in general through his work on epidemiological surveillance, animal health economics, the use of information technology in epidemiological decision-making and his contribution to capacity building of veterinary services around the world.

Professor Morris has been influential in the development of evidence-based policy and disease control in many countries as well as for international organizations including World Organization for Animal Health (OIE), the UN Food and Agriculture Organization (FAO) and the World Bank. He has had a significant role in having veterinary epidemiology recognized as a scientific discipline within veterinary science with direct impact on animal disease risk management. In doing so, he has made an enormous contribution towards the global community being able to more effectively deal with new and emerging disease threats associated with the continuing globalization of trade in animals and animal-derived products. His networking and contributions have helped change standards and legislation to incorporate evidence- and risk-based approaches. This has led to improved policies from which farmers and consumers continue to benefit in many countries. Between 1969 and the present, Dr Morris has been involved internationally in advice and consultancy to governments, international organizations, research institutions and industry bodies on animal health and human implications of animal diseases.

## 2014 CRWAD PROGRAM - BY THE DAY

ABSTRACTS AVAILABLE AT THE ON-LINE MEETING PLANNER AND ITINERARY BUILDER  
<http://www.cvmbs.colostate.edu/mip/crwad/>

### **Speaker Ready Room: (Section meeting rooms are listed inside front cover)**

Streeterville Room (2<sup>nd</sup> floor) is available on Sunday, Dec. 7 - Monday, Dec. 8

### **POSTER INFORMATION - Poster Sessions I & II - Grand Ballroom III, 7<sup>th</sup> Floor**

**SUNDAY POSTER PRESENTERS:** December 7, 6:30 - 8:00 PM.

Poster boards will be available for poster assembly by 4 PM Sunday. Posters for the Bacterial Pathogenesis, Companion Animal Epidemiology, Epidemiology and Animal Health Economics, and Pathobiology of Enteric and Foodborne Pathogens Sections will be presented Sunday from 6:30-8:00 PM. Please remove your posters by 10:00 AM Monday.

**MONDAY POSTER PRESENTERS:** December 8, 5:00 - 6:30 PM

Poster boards will be available for poster assembly by noon Monday. Posters for the Ecology and Management of Foodborne Agents, Immunology, Respiratory Diseases, Vector-Borne and Parasitic Diseases, and Virology Sections will be presented Monday from 5:00-6:30 PM. Please remove your posters immediately upon completion of Poster Session II, by 6:30 PM.

Poster Boards are 4 ft tall x 8 ft wide; one poster per side; must furnish your own tacks.

### **NOTICE:**

Poster Presenters must be with their competition entry posters for possible judge interviews. Poster Presenters (and oral presenters) must wear their name badge during their presentation and must be registered for the CRWAD meeting.

The Graduate Student Competition Awards will be presented during the Tuesday Business Meeting. All students entered in the competition are invited and encouraged to attend the Business Meeting.

## PROGRAM - BY THE DAY

### **Symposiums**

Saturday, Dec. 6, 8AM - 5:00 PM - NC1202 Enteric Diseases of Food Animals

Sunday, Dec. 7, 8AM - 12:00 PM - NC1202 Enteric Diseases of Food Animals

Sunday, Dec. 7, 8 AM - 11:00 PM - AVEPM Workshop

Sunday - Dec. 7, 11:30 AM - 5 PM - AVEPM Symposium Program - Open Attendance

Sunday, Dec. 7, 1 PM - 5:00 PM - NC229 PRRSV Meeting

Monday, Dec. 8, ACVM Mini-Symposium to be presented within the Bacterial Pathogenesis Section of the CRWAD

Tuesday, Dec. 9, AAVI Mini-Symposium: "The Analysis and the Role of the Microbiome in Immunology," to be presented within the Immunology Section of the CRWAD

### **CRWAD Meeting Begins Sunday (evening):**

**Notice: Section meeting rooms are listed inside front cover**

Sunday - Dec. 7 - 6:00-8:00 PM - Kick-Off CRWAD Reception and Poster Session I

Monday - Dec. 8, 8:00 AM - CRWAD Sections Oral Presentations begin in eight separate rooms

Monday - Dec. 8, 5:00 PM - 6:30 PM - Poster Session II

Tuesday - Dec. 9, 8:00 AM - CRWAD Sections Oral Presentations begin in separate rooms

Tuesday - Dec. 9, 11:45 AM - CRWAD Business Meeting, Student Competition Awards, Dedication and Other Awards

Time	Oral #	Section	Monday-By-The-Day Title
8:00	001	Bacterial Pathogenesis	Identification of a Type II Toxin-antitoxin System in <i>Campylobacter jejuni</i>
8:00	031	Companion Animal Epidemiology	Scrotal castration as a safe and effective means of male canine sterilization
8:00	062	Epidemiology & Animal Health Economics	Estimating the Number of Human Cases of Ceftiofur-Resistant <i>Salmonella enterica</i> serovar Heidelberg in Québec and Ontario, Canada (2003-2011)
8:00	095	Immunology	Evaluation of porcine epidemic diarrhea virus transmission and the immune response in growing pigs
8:00	125	Pathobiology of Enteric and Foodborne Pathogens	Most Prevalent Poultry-associated <i>Salmonella</i> SeroTypes differ in their susceptibility to widely used carcass sanitizer, chlorine
8:00	137	Respiratory Diseases	Effect of pretreatment on detection of PRRSV in oral fluid by qRT-PCR assay
8:00	153	Vector-Borne and Parasitic Diseases	Real-time PCR Assay Validation for Detecting <i>Rickettsia rickettsii</i> infections in dogs and ticks
8:15	002	Bacterial Pathogenesis	A single nucleotide change in <i>mutY</i> leads to the increased emergence of fluoroquinolones resistant mutants in <i>Campylobacter jejuni</i>
8:15	032	Companion Animal Epidemiology	Factors associated with hematuric struvite crystalluria in cats evaluated at general care veterinary hospitals in the United States (2007-2011)
8:15	063	Epidemiology & Animal Health Economics	Extended-spectrum cephalosporin resistant nontyphoidal <i>Salmonella</i> recovered from clinical human infections in Ohio, USA.
8:15	096	Immunology	In vitro evaluation of serological cross-reactivity and cross-neutralization between the U.S. PEDV original and variant strains
8:15	126	Pathobiology of Enteric and Foodborne Pathogens	A randomized trial to assess whether enrofloxacin metaphylaxis for bovine respiratory disease affects fecal shedding of <i>Salmonella</i> and <i>Campylobacter</i> in feedlot cattle
8:15	138	Respiratory Diseases	Vaccination mitigates the negative impact PRRSV infection has on the pharmacokinetics of ceftiofur crystalline free acid in pigs.
8:15	154	Vector-Borne and Parasitic Diseases	Molecular prevalence of <i>Theileria</i> spp. in ruminants from nine provinces of China
8:30	003	Bacterial Pathogenesis	Transcriptomic responses of a <i>Campylobacter jejuni</i> strain associated with sheep abortion to sheep whole blood and identification of a novel abortion-inducing factor
8:30	033	Companion Animal Epidemiology	Prevalence and association with Feline Upper Respiratory Disease severity for the detection of select pathogens and risk factors in Midwestern animal shelter cats

Time	Oral #	Section	Monday-By-The-Day Title
8:30	064	Epidemiology & Animal Health Economics	Understanding the occurrence of Escherichia coli O157:H7 super-shedding infections in feedlot cattle
8:30	097	Immunology	Relationship between maternal immune status and neonatal protection against PEDV infection
8:30	127	Pathobiology of Enteric and Foodborne Pathogens	Characterization of a Putative Hemolysin of Campylobacter jejuni for Potential Use in Vaccination of Poultry.
8:30	139	Respiratory Diseases	Genetic diversity analysis of genotype 2 porcine reproductive and respiratory syndrome viruses emerging in recent years in China
8:30	155	Vector-Borne and Parasitic Diseases	Portable insulated isothermal RT-PCR (iiRT-PCR) assay for sensitive and specific detection of bluetongue virus
8:45	004	Bacterial Pathogenesis	Environmental persistence and biofilm formation of hypermucoid and non-hypermucoid Klebsiella pneumoniae
8:45	034	Companion Animal Epidemiology	An insulated isothermal PCR-POCKIT™ method for rapid and sensitive detection of canine parvovirus at the point of care
8:45	065	Epidemiology & Animal Health Economics	Effect of feeding preweaned dairy calves raw milk with residual concentrations of antimicrobials on the resistance of commensal fecal Escherichia coli.
8:45	098	Immunology	Interaction of interferons and mTOR signaling underlying PRRSV infection
8:45	128	Pathobiology of Enteric and Foodborne Pathogens	Plasmids of enteric bacterial pathogens: past, present, and future challenges
8:45	140	Respiratory Diseases	In vivo targeting of porcine reproductive and respiratory syndrome virus antigen through porcine DC-SIGN to dendritic cells elicits antigen-specific CD4 T cell immunity in pigs
8:45	156	Vector-Borne and Parasitic Diseases	Surveillance of ecto- and endoparasitism in northern Mississippi canine shelter populations
9:00	005	Bacterial Pathogenesis	Characterization of Glucosamine-6-Phosphate Synthase (GlmS) mutant of Salmonella: Effect on Biology and Pathogenesis of the Organism
9:00	035	Companion Animal Epidemiology	Molecular epidemiological analysis of MRSP environmental contamination in a veterinary hospital: Looking beyond infections and treatments
9:00	066	Epidemiology & Animal Health Economics	Longitudinal study of nasal carriage of Staphylococcus aureus in swine veterinarians and its implications for health
9:00	099	Immunology	Predicting vaccine efficacy for food animals using the Epitope Content Comparison (EpiCC) tool: Application to PRRSV
9:00	141	Respiratory Diseases	Neuraminidase inhibiting (NI) antibodies induced in pigs by experimental influenza A virus vaccines

Time	Oral #	Section	Monday-By-The-Day Title
9:00	157	Vector-Borne and Parasitic Diseases	Evaluation of <i>Dermacentor variabilis</i> for the incidence of pathogens causing diseases in animals and humans
9:15	006	Bacterial Pathogenesis	Human specific virulence factors absent in LA-MRSA ST5 strains isolated from pigs, swine facilities, and humans with swine contact.
9:15	036	Companion Animal Epidemiology	Use of patient body temperatures in surveillance for healthcare-associated infections in a veterinary hospital.
9:15	067	Epidemiology & Animal Health Economics	Bile salt hydrolase: a microbiome target for enhanced animal health
9:15	100	Immunology	Evaluation of cross-protection in Foster <sup>TM</sup> PRRSV vaccinated conventional swine challenged with a contemporary, heterologous lineage 9 PRRSV field isolate.
9:15	142	Respiratory Diseases	Influenza transmission within coordinated swine production systems
9:15	158	Vector-Borne and Parasitic Diseases	Investigation into horn fly burden susceptibility in Holstein heifers
9:45	129	Pathobiology of Enteric and Foodborne Pathogens	Pooling of immunomagnetic separation beads does not affect sensitivity of detection of seven serogroups of Shiga toxin-producing <i>Escherichia coli</i> in cattle feces
10:00	007	Bacterial Pathogenesis	Global control of virulence and survival of <i>Mycobacterium avium</i> subsp. <i>paratuberculosis</i> .
10:00	037	Companion Animal Epidemiology	Non-fatal injury occurrence in southern California Thoroughbred racehorses 2009-2010
10:00	068	Epidemiology & Animal Health Economics	Investigating the efficacy of antimicrobial metaphylaxis in finishing pigs
10:00	101	Immunology	Innovative polymeric adjuvant for PCV2 vaccination
10:00	130	Pathobiology of Enteric and Foodborne Pathogens	Transcriptional profiling of <i>Salmonella</i> Enteritidis strains identifies genes consistently highly expressed in biologically relevant microenvironments
10:00	143	Respiratory Diseases	Co-circulation of two distinct genetic and antigenic lineages of proposed influenza D virus in cattle
10:00	159	Vector-Borne and Parasitic Diseases	Bartonellosis: One Health Perspectives on an Emerging Infectious Disease.
10:15	008	Bacterial Pathogenesis	Overexpression of catalytically inactive dimethyl adenosine transferase (KsgA) unveils contribution of KsgA to <i>Salmonella</i> Enteritidis physiology and virulence
10:15	038	Companion Animal Epidemiology	Factors associated with long-term survival of geriatric horses in the U.S.
		(continued)	

## 2014 Program By-The-Day

Time	Oral #	Section	Monday-By-The-Day Title
10:15	069	Epidemiology & Animal Health Economics	Role of direct and indirect transmission of different PRRSV genotypes within and between swine production systems in the US
10:15	102	Immunology	Mucosal correlates of cross-protection for live-attenuated influenza virus vaccines in pigs.
10:15	131	Pathobiology of Enteric and Foodborne Pathogens	Identification and characterization of immune-modulatory CpG motifs of Salmonella
10:15	144	Respiratory Diseases	An RSV fusion inhibitor is an effective treatment for bovine respiratory syncytial virus infection of calves
10:30	039	Companion Animal Epidemiology	Canine B-cell chronic lymphocytic leukemia shows strong breed-specific risk
10:30	070	Epidemiology & Animal Health Economics	Disease investigation using data from a PRRS area regional control and elimination (ARC&E) project in Ontario, Canada.
10:30	103	Immunology	PLGA-Nanoparticle entrapped swine influenza virus peptides vaccine induces epitope specific cell-mediated immune response in pigs
10:30	132	Pathobiology of Enteric and Foodborne Pathogens	Diversity and distribution of a novel swine dysentery pathogen "Brachyspira hampsonii"
10:30	145	Respiratory Diseases	Improving case definitions for BRD treatment with an algorithm based analysis of lung auscultation
10:45	009	Bacterial Pathogenesis	Towards an understanding of Salmonella persistence in pigs.
10:45	040	Companion Animal Epidemiology	Multicenter case-control investigation of risks associated with development of canine lymphoma in North America: 18,826 cases (1990-2009)
10:45	071	Epidemiology & Animal Health Economics	Behavioral aspects of oral fluid sample collection
10:45	104	Immunology	Recovery and Stability of DNA and Protein Antigens Formulated with VaxLiant Adjuvants Utilizing in vitro and in vivo Testing
10:45	133	Pathobiology of Enteric and Foodborne Pathogens	The humoral immune response of pigs and horses against the vaccine of Lawsonia intracellularis.
10:45	146	Respiratory Diseases	Identification of pens at high risk for BRD with an algorithm based analysis of thoracic auscultation at post arrival processing
10:45	160	Vector-Borne and Parasitic Diseases	Rickettsia felis in China
11:00	041	Companion Animal Epidemiology	Differences in the geographic distribution of B-cell and T-zone lymphomas in Golden retrievers in the United States
11:00	072	Epidemiology & Animal Health Economics	Influenza H1N1 and H3N2 co-infection in pigs afterweaning.
11:00	105	Immunology	Bovine ocular and systemic immune responses to an intranasal recombinant Moraxella bovis cytotoxin subunit vaccine adjuvanted with polyacrylic acid

## 2014 Program By-The-Day

Time	Oral #	Section	Monday-By-The-Day Title
11:00	134	Pathobiology of Enteric and Foodborne Pathogens	Temperature-dependent conjugative gene transfer in <i>Campylobacter jejuni</i>
11:00	147	Respiratory Diseases	Acute phase proteins in naturally occurring respiratory disease of feedlot cattle: a novel approach to diagnosis.
11:15	106	Immunology	Effect of VaxLiant adjuvants on efficacy of mucosal administration of plant-derived Newcastle Disease vaccines in chickens
11:15	135	Pathobiology of Enteric and Foodborne Pathogens	Determination of the minimum infectious dose of porcine epidemic diarrhea virus in neonatal and weaned pigs
11:15	148	Respiratory Diseases	Cytokine profiles from shipping through sickness and recovery in cattle either mass-medicated with gamithromycin or sham-treated
1:30	010	Bacterial Pathogenesis	Brucellosis in animal reservoirs in the united states: challenges and opportunities
1:30	021	Biosafety and Biosecurity	Prevalence, genotypes, and risk factors for <i>Clostridium perfringens</i> among Ontario broiler chicken flocks
1:30	043	Ecology and Management of Foodborne Agents	<i>Campylobacter jejuni</i> isolated from cattle in Michigan: genetic diversity, antimicrobial resistance, and the impact on public health
1:30	073	Epidemiology & Animal Health Economics	Smartphone applications for veterinary data collection in Malaysia: feasibility and data usage in animal disease surveillance
1:30	107	Immunology	Challenges with Poultry Immunology and Disease Research: Immune reagent development and maintaining genetic breeds of poultry
1:30	161	Viral Pathogenesis	A unique mechanism of protein-directed trans-activation of ribosomal frameshifting in arteriviruses
1:45	022	Biosafety and Biosecurity	Seroprevalence and risk factors for <i>Coxiella burnetii</i> exposure in Ontario sheep and goats
1:45	044	Ecology and Management of Foodborne Agents	An evaluation of the impact of litter chemical amendments on reducing <i>Campylobacter jejuni</i> in broilers
1:45	074	Epidemiology & Animal Health Economics	Characterization of the network of live fish movements in the Irish salmon farming industry and implications for disease prevention and control
1:45	162	Viral Pathogenesis	Regulatory role of the SAP-like motif of PRRSV nsp1 $\beta$ protein for innate immune response
2:00	011	Bacterial Pathogenesis	Bovine tuberculosis eradication: Update and emerging issues
2:00	023	Biosafety and Biosecurity	Identifying cattle farms and areas for risk-based surveillance for bovine tuberculosis in Minnesota
		(continued)	

Time	Oral #	Section	Monday-By-The-Day Title
2:00	045	Ecology and Management of Foodborne Agents	Summer and winter prevalence of O26, O45, O103, O111, O121, O145 and O157 Shiga toxin-producing <i>Escherichia coli</i> (STEC) in feces of feedlot cattle
2:00	075	Epidemiology & Animal Health Economics	A qualitative risk model for animal welfare concerns due to movement restrictions during a classical swine fever (CSF) outbreak
2:00	163	Viral Pathogenesis	The PRRSV-mediated inhibition of IFN $\alpha$ production by pig alveolar macrophages occurs at the post-transcriptional level via the activation of eIF-2 $\alpha$
2:15	024	Biosafety and Biosecurity	Detection of <i>Salmonella enterica</i> in the dairy environment using a commercially available lateral flow immunoassay.
2:15	046	Ecology and Management of Foodborne Agents	Feedlot- and pen-level prevalence of Shiga toxin-producing <i>Escherichia coli</i> in feces of commercial feedlot cattle
2:15	076	Epidemiology & Animal Health Economics	The Alberta Veterinary Surveillance Network: Creating a tool to track cattle diseases and movements in Alberta, Canada
2:15	108	Immunology	Propagation of feline respiratory epithelial cells at the air-liquid interface - an in vitro model to study feline herpesvirus 1 in cats
2:15	164	Viral Pathogenesis	Development of a synthetic porcine reproductive and respiratory syndrome virus strain that confers broader cross-protection
2:30	025	Biosafety and Biosecurity	Improved characterization of <i>Salmonella enterica</i> shedding among reptile patients at the James L. Voss Veterinary Teaching Hospital
2:30	047	Ecology and Management of Foodborne Agents	Prevalence of targeted enterohemorrhagic <i>Escherichia coli</i> in culled dairy cows
2:30	077	Epidemiology & Animal Health Economics	Using scan statistics to explore the relative performance of dead birds and mosquito pools in surveillance for West Nile virus in Ontario, 2002-2008.
2:30	109	Immunology	The role of interferon in clearing primary Bovine Herpesvirus-1 (BHV-1) infections
2:30	165	Viral Pathogenesis	Characterization of entry events during bile acid-mediated porcine enteric calicivirus replication
3:00	012	Bacterial Pathogenesis	Arbovirus research and epizootic hemorrhagic disease
3:00	026	Biosafety and Biosecurity	Biosecurity, a biography: ten years of hard lessons.
3:00	048	Ecology and Management of Foodborne Agents	Quantification of six non-O157 <i>E. coli</i> serogroups in cattle feces by spiral plating method
3:00	078	Epidemiology & Animal Health Economics	A meta-analysis of the effects of feeding active dry yeast of <i>Saccharomyces cerevisiae</i> , on milk production of lactating dairy cows
		(continued)	



Time	Oral #	Section	Monday-By-The-Day Title
3:00	110	Immunology	Late-gestational nutrient restriction affects immune responsiveness of offspring in beef cattle.
3:00	149	Respiratory Diseases	Identification and characterization of bovine rhinitis viruses in bovine respiratory disease clinical specimens
3:00	166	Viral Pathogenesis	The spike protein furin cleavage site in feline coronavirus is not the sole determinant of conversion to feline infectious peritonitis virus
3:15	049	Ecology and Management of Foodborne Agents	Modeling the intestinal concentrations of antimicrobials in animals
3:15	079	Epidemiology & Animal Health Economics	Dairy cattle management factors that influence on-farm density of European starlings ( <i>Sturnus vulgaris</i> ) in Ohio, 2007 - 2009
3:15	111	Immunology	Immune suppression in BLV-infected dairy cattle
3:15	150	Respiratory Diseases	Characterization of Biofilm Formation by <i>Pasteurella multocida</i>
3:15	167	Viral Pathogenesis	Assessment of viremia and tissue distribution of porcine epidemic diarrhea virus in weaned pigs after experimental infection
3:30	013	Bacterial Pathogenesis	Has changing animal husbandry practices changed the ecology of influenza?
3:30	050	Ecology and Management of Foodborne Agents	Effect of heifer-raising practices on <i>E. coli</i> antimicrobial resistance and <i>Salmonella</i> prevalence among heifer fecal pats.
3:30	080	Epidemiology & Animal Health Economics	Spatial clustering of bovine tuberculosis outbreaks in Uruguay.
3:30	112	Immunology	Effects of conditioned media from <i>Histophilus somni</i> infected bovine brain endothelial cells on fibrin deposition and Factor Xa activity of bovine neutrophils
3:30	151	Respiratory Diseases	Septic pleuropneumonia in 41 horses (2000 - 2014)
3:30	168	Viral Pathogenesis	Sequencing analysis of recently outbreaked porcine epidemic diarrhea virus in vietnam
3:45	027	Biosafety and Biosecurity	Evaluation of POKIT, field deployable technology, for molecular based detection of Foot and Mouth Disease virus, Classical Swine Fever virus and African Swine Fever virus.
3:45	051	Ecology and Management of Foodborne Agents	Using metagenomics to unlock the ecology of antimicrobial resistance in cattle production systems
3:45	081	Epidemiology & Animal Health Economics	The effect of morbidity on weaning weight of beef calves
3:45	113	Immunology	Expression of inflammation-associated genes in circulating leukocytes and activity of indoleamine-2,3-dioxygenase in dairy cattle with acute puerperal metritis and bacteremia.
		(continued)	

2014 Program By-The-Day

Time	Oral #	Section	Monday-By-The-Day Title
3:45	152	Respiratory Diseases	Regulation of interferon-gamma gene expression in foals and its relationship to susceptibility to <i>Rhodococcus equi</i> .
3:45	169	Viral Pathogenesis	Pathogenesis of US porcine deltacoronavirus strains FD22 and FD100 in gnotobiotic pigs.
4:00	028	Biosafety and Biosecurity	Using social network analysis to understand epidemic potential in equine populations: a pilot study.
4:00	052	Ecology and Management of Foodborne Agents	Enterobacteriaceae producing extended spectrum beta-lactamases from wild birds on Ohio dairies.
4:00	082	Epidemiology & Animal Health Economics	Performance of FAMACHA scores for detecting anemia in sheep
4:00	114	Immunology	Oxylipid profiles in biological samples of dairy cows with coliform mastitis.
4:00	170	Viral Pathogenesis	Development and validation of an indirect porcine deltacoronavirus (PDCoV) anti-IgG ELISA based on the S1 portion of the spike protein and confirmation that PDCoV infection in U.S. pigs is low and has been present since 2010
4:15	029	Biosafety and Biosecurity	Assessing spatial dispersal of pathogens around human settlements
4:15	053	Ecology and Management of Foodborne Agents	Prevalence and characteristics of <i>Salmonella</i> found on the paws and in the feces of free-ranging raccoons ( <i>Procyon lotor</i> ) in southern Ontario, Canada.
4:15	083	Epidemiology & Animal Health Economics	Respiratory disease outbreak detection within a population of free-living chimpanzees ( <i>Pan troglodytes schweinfurthii</i> )
4:15	115	Immunology	Bovine neutrophils produce neutrophil extracellular traps (NETs) in response to <i>Salmonella</i> serotype Typhimurium
4:30	030	Biosafety and Biosecurity	Survey of Iowa residential bats for influenza viruses

Time	Oral #	Section	Tuesday-By-The-Day Title
8:00	014	Bacterial Pathogenesis	Mannheimia haemolytica biofilm formation on bovine respiratory epithelial cells
8:00	084	Epidemiology & Animal Health Economics	The Symbiology of Epidemiologic Pursuits of Academia and Government.
8:00	116	Immunology	The Microbiota-Gut-Brain Axis: Increasing recognition of its role in health and disease
8:00	172	Viral Pathogenesis	African swine fever virus (ASFV) rp30 ELISA detects antibody in serum and/or oral fluid specimens
8:15	015	Bacterial Pathogenesis	Characterization of a novel isolate of Leptospira associated with the greater white-toothed shrew (Crocidura russula), an invasive mammalian species in Ireland.
8:15	173	Viral Pathogenesis	Acute infection with bovine viral diarrhea virus causes depletion of WC1+ $\gamma\delta$ T cells in lymphoid tissues in beef calves
8:30	016	Bacterial Pathogenesis	Complete genome sequencing and analysis of the red blood cell pathogen of llamas and alpacas, 'Candidatus Mycoplasma haemolamae'.
8:30	117	Immunology	Recovery of the gut microbiota is disturbance-dependent
8:30	174	Viral Pathogenesis	The bovine immunodeficiency virus Rev protein: characterization of the multimerization domain using the bimolecular fluorescence complementation (BiFC) technology
8:45	017	Bacterial Pathogenesis	Insulated isothermal PCR assay for the detection of Taylorella equigenitalis
8:45	085	Epidemiology & Animal Health Economics	Mannheimia haemolytica in feedlot cattle: associations with antimicrobial use, resistance and health outcomes
8:45	118	Immunology	Developing tools to investigate the swine-associated butyrate-producing microbiota and its relationship to Salmonella shedding phenotype
8:45	175	Viral Pathogenesis	Efficacy of M2e-based vaccine in murine, avian and swine models
9:00	018	Bacterial Pathogenesis	An internal control improves the detection of Brucella canis in diagnostic samples analyzed by a triplex real-time PCR assay
9:00	054	Ecology and Management of Foodborne Agents	Potential transfer of antimicrobial resistance (AMR) Salmonella and Staphylococcus sciuri after application of swine manure in the environment
9:00	086	Epidemiology & Animal Health Economics	Reporting guidelines for observational studies in veterinary medicine: STROBE-Vet
9:00	119	Immunology	Tailoring probiotics as immunomodulators to enhance neonatal mucosal immunity to rotavirus (RV) vaccines or alleviate RV diarrhea: Evaluation in a neonatal gnotobiotic piglet model.

## 2014 Program By-The-Day

Time	Oral #	Section	Tuesday-By-The-Day Title
9:00	176	Viral Pathogenesis	Potential of endogenously produced and exogenously administered interferon as adjuvant for influenza vaccine in animals
9:15	019	Bacterial Pathogenesis	Actinobacillus pleuropneumoniae (APP) ApxIV toxin antibody ontogeny in serum, oral fluid and fecal specimens from animals inoculated under experimental conditions.
9:15	055	Ecology and Management of Foodborne Agents	Risk factors for antimicrobial resistance of Escherichia coli isolates from Ontario broiler chicken flocks at chick placement: A comparison of three production system types
9:15	087	Epidemiology & Animal Health Economics	Mathematical disease transmission models for livestock populations: A scoping review
9:15	177	Viral Pathogenesis	Poly I:C adjuvanted inactivated swine influenza vaccine induces heterologous protective immunity in pigs
10:00	056	Ecology and Management of Foodborne Agents	Predictors for contamination of informally traded ready-to-eat (RTE) chicken with generic (Biotype I) Escherichia coli
10:00	088	Epidemiology & Animal Health Economics	Does exercise-induced pulmonary hemorrhage affect career longevity and performance among South African Thoroughbred racehorses?
10:00	120	Immunology	Directed Evolution Of An Adeno-Associated Virus Library In Vivo Pig Airway.
10:00	178	Viral Pathogenesis	Viral Pathogenesis: Lessons Learned from Newcastle Disease Virus.
10:15	057	Ecology and Management of Foodborne Agents	Salmonella phenotypic and genotypic diversity in finishing swine
10:15	089	Epidemiology & Animal Health Economics	Human Q fever: seroprevalence and exploration of risk factors for Coxiella burnetii exposure in small ruminant farm workers and veterinarians/veterinary students
10:30	058	Ecology and Management of Foodborne Agents	Oral Salmonella challenge and subsequent uptake by the peripheral lymph nodes in calves
10:30	090	Epidemiology & Animal Health Economics	Rare disease epidemiology: results from the national scrapie prevalence study
10:30	121	Immunology	Evaluating the metagenome of nasal samples from cattle with bovine respiratory disease complex (BRDC)
10:45	059	Ecology and Management of Foodborne Agents	High throughput environmental testing for Salmonella sp. using Matrix-Assisted Laser Desorption/Ionization-Time of Flight MALDI-TOF) technology
10:45	091	Epidemiology & Animal Health Economics	Epidemiology of Salmonella spp. among feral pigs in Texas
		(continued)	

2014 Program By-The-Day

Time	Oral #	Section	Tuesday-By-The-Day Title
10:45	179	Viral Pathogenesis	Rescue and characterization of a new re-assortant influenza virus strain H5N1
11:00	060	Ecology and Management of Foodborne Agents	Designing a risk communication strategy for health hazards posed by traditional slaughter of goats in Tshwane, South Africa
11:00	092	Epidemiology & Animal Health Economics	Risk mapping of Avian influenza in California using Multiple criteria decision analysis.
11:00	122	Immunology	Evaluation of changes in VapA-specific IgG and IgG subclasses over time to identify foals with Rhodococcus equi pneumonia
11:00	180	Viral Pathogenesis	Naturally truncated NS gene of H3N8 equine influenza virus attenuates the virulence of the A/Puerto Rico/8/34 virus
11:15	061	Ecology and Management of Foodborne Agents	Evidence from bioassays that commercial spray drying processes are effective at inactivating porcine epidemic diarrhea virus
11:15	093	Epidemiology & Animal Health Economics	Within-farm spread of highly pathogenic avian influenza in Korean outbreaks of H5N1 and H5N8 virus types
11:15	123	Immunology	The effect of passively-acquired antibodies on Lawsonia intracellularis infection and immunity in the horse
11:15	181	Viral Pathogenesis	Role of Ebola virus matrix protein in regulating cellular innate immune response

# **POSTER PROGRAM**

# BACTERIAL PATHOGENESIS POSTERS

Poster Session I - Sunday 6:30-8:00 PM - Grand Ballroom Salon III - 7th floor

Section Leader: Gireesh Rajashekara

Poster assembly begins at 4 PM Sunday. Please remove your posters by 10:00 AM Monday.

Poster Presenters must be with their competition entry posters for possible judge interviews.

Name badge are required.

No.	Title	Authors
001P	Seroprevalence of <i>Yersinia enterocolitica</i> in different mammalian hosts at a nonhuman primate research facility	<b>S.J. Rostad</b> <sup>1</sup> , S. Francis <sup>1</sup> , J. Berezowski <sup>1</sup> , J. Berezowski <sup>2</sup> , A. Beierschmitt <sup>3</sup> , M. McCoy <sup>1</sup> , A. Loftis <sup>1</sup> , D. Boruta <sup>1</sup> , O. Illanes <sup>1</sup> , D. Recinos <sup>1</sup> , M. Arauz <sup>1</sup> , D. Spencer <sup>1</sup> , E. Soto <sup>1</sup> ; <sup>1</sup> Ross University School of Veterinary Medicine, Basseterre, Saint Kitts and Nevis, <sup>2</sup> Veterinary Public Health Institute, University of Bern, Bern, Switzerland, <sup>3</sup> Behavioural Science Foundation, Estridge Estate, Saint Kitts and Nevis
002P	Phylogenetic analysis of <i>Dermatophilus congolensis</i> isolated from naturally infected cattle in Abeokuta and Ilorin, Nigeria.	<b>F.S. Oladunni</b> <sup>1</sup> , A.O. Talabi <sup>2</sup> , M.I. Takeet <sup>3</sup> , E.O. Ojo <sup>3</sup> , M.A. Oyekunle <sup>3</sup> ; <sup>1</sup> Veterinary Microbiology, University of Ilorin, Ilorin, Nigeria, <sup>2</sup> Veterinary Medicine, Federal University of Agriculture, Abeokuta, Nigeria, <sup>3</sup> Veterinary Microbiology and Parasitology, Federal University of Agriculture, Abeokuta, Nigeria
003P	MALDI-TOF as a novel detection method for <i>Clostridium difficile</i> toxins	<b>K. Hammac</b> <sup>1</sup> , K.B. Ray <sup>2</sup> , C.R. Wilson <sup>1</sup> ; <sup>1</sup> Indiana Animal Disease Diagnostic Laboratory, Purdue University, West Lafayette, IN, USA, <sup>2</sup> College of Veterinary Medicine, Purdue University, West Lafayette, IN, USA
004P	Serological investigations of <i>Lawsonia intracellularis</i> in horses on breeding farms in Japan	D. Miyayama <sup>1</sup> , R. Uemura <sup>1</sup> , Y. Sasaki <sup>2</sup> , H. Niwa <sup>3</sup> , T. Higuchi <sup>4</sup> , T. Harada <sup>5</sup> , <b>M. Sueyoshi</b> <sup>6</sup> ; <sup>1</sup> Dept. of Veterinary Science, University of Miyazaki, Miyazaki, Japan, <sup>2</sup> Org. for Promotion of Tenure-track, University of Miyazaki, Miyazaki, Japan, <sup>3</sup> Equine Research Institute, The Japan Racing Association, Tochigi, Japan, <sup>4</sup> Hidaka Agricultural Mutual Relief Association, Hokkaido, Japan, <sup>5</sup> Hidaka Livestock Hygiene Service Center, Hokkaido, Japan, <sup>6</sup> The Center for Animal Disease Control, University of Miyazaki, Miyazaki, Japan.

# COMPANION ANIMAL EPIDEMIOLOGY POSTERS

Poster Session I - Sunday 6:30-8:00 PM - Grand Ballroom Salon III - 7th floor

Section Leaders: Audrey Ruple-Czerniak and Laura Hungerford

Poster assembly begins at 4 PM Sunday. Please remove your posters by 10:00 AM Monday.

Poster Presenters must be with their competition entry posters for possible judge interviews.

Name badge is required.

No.	Title	Authors
005P	Effect of dog body condition score on lipoprotein cholesterol and triglyceride concentrations; effect of recommended fasting duration on sample concentrations	<b>S. Usui</b> <sup>1</sup> , H. Yasuda <sup>2</sup> , Y. Koketsu <sup>1</sup> ; <sup>1</sup> School of Agriculture, Meiji University, Kawasaki, Japan, <sup>2</sup> Spectrum Lab Japan, Tokyo, Japan.
006P	Risk factors associated with obese or overweight dogs visited at Japanese private veterinary clinics	<b>S. Usui</b> <sup>1</sup> , H. Yasuda <sup>2</sup> , Y. Koketsu <sup>1</sup> ; <sup>1</sup> School of Agriculture, Meiji University, Kawasaki, Japan, <sup>2</sup> Spectrum Lab Japan, Tokyo, Japan.
007P	Multidrug resistant infections in dogs in a veterinary teaching hospital: risk factors for fecal carriage.	S. Stevens <sup>1</sup> , L. Quan <sup>1</sup> , R. McClanahan <sup>1</sup> , L.P. Jones <sup>2</sup> , <b>M.A. Davis</b> <sup>2</sup> ; <sup>1</sup> Veterinary Clinical Sciences, Washington State University, Pullman, WA, USA, <sup>2</sup> School for Global Animal Health, Washington State University, Pullman, WA, USA.
008P	Extended-spectrum beta-lactamase producing enterobacteriaceae recovered from healthy dogs.	<b>D.A. Mathys</b> <sup>1</sup> , D.F. Mollenkopf <sup>1</sup> , J.B. Daniels <sup>2</sup> , T.E. Wittum <sup>1</sup> ; <sup>1</sup> Department of Veterinary Preventative Medicine, College of Veterinary Medicine, Ohio State University, Columbus, OH, USA, <sup>2</sup> Department of Veterinary Clinical Sciences, College of Veterinary Medicine, Ohio State University, Columbus, OH, USA.
009P	Exposure factors associated with side effects in dogs after treatment with Chinese herbs	<b>C. Yu</b> <sup>1</sup> , H. Xie <sup>1</sup> , L. Trevisanetto <sup>2</sup> , J. Shmalberg <sup>1</sup> , J. Hernandez <sup>1</sup> ; <sup>1</sup> College of Veterinary Medicine, University of Florida, Gainesville, FL, USA, <sup>2</sup> Chi Institute, Reddick, FL, USA.
010P	Creation of a bioarchive to the prevalent brucella strains in Egypt	<b>H.I. Hosen</b> <sup>1</sup> , A.E. Sayour <sup>2</sup> , R.A.A. Azzam <sup>1</sup> , A. Menshaw <sup>1</sup> ; <sup>1</sup> Veterinary Medicine, Beni Suef University, Beni Suef, Egypt, <sup>2</sup> Brucellosis, AHRI, AHRI, Egypt.



# **ECOLOGY AND MANAGEMENT OF FOODBORNE AGENTS POSTERS**

**Poster Session II - Monday 5:00 - 6:30 PM - Grand Ballroom Salon III - 7th floor**

**Section Leader: Yvette Johnson-Walker**

Poster assembly begins at noon Monday. Please remove your posters by 6:30 PM Monday.

Poster Presenters must be with their competition entry posters for possible judge interviews.

Name badge is required.

No.	Title	Authors
<b>011P</b>	Specificity analysis and detection software evaluation of PathoProof <sup>®</sup> Mastitis PCR Assay	<b>L. Marshall Lund<sup>1</sup></b> , K. Harmon <sup>2</sup> , T. Frana <sup>1</sup> ; <sup>1</sup> Veterinary Diagnostic and Production Animal Medicine, Iowa State University, Ames, IA, USA, <sup>2</sup> Veterinary Diagnostic Laboratory, Iowa State University, Ames, IA, USA
<b>012P</b>	Association between temperament and E. coli O157:H7 shedding in Brahman calves.	<b>E.V. Gart<sup>1</sup></b> , T.H. Welsh, Jr <sup>2</sup> , R.D. Randel <sup>3</sup> , R.C. Vann <sup>4</sup> , S.D. Lawhon <sup>1</sup> ; <sup>1</sup> VTPB, Texas A&M, College Station, TX, USA, <sup>2</sup> Animal Science, Texas A&M, College Station, TX, USA, <sup>3</sup> Texas A&M AgriLife Research, Overton, TX, USA, <sup>4</sup> MAFES-Brown Loam, Mississippi State University, Raymond, MS, USA
<b>013P</b>	Stress and non-typhoidal <i>Salmonella enterica</i> : quantifying the impact of stocking density in dairy cattle.	<b>L.M. Muñoz Vargas<sup>1</sup></b> , J. Pempek <sup>2</sup> , K. Proudfoot <sup>1</sup> , M. Eastridge <sup>2</sup> , G. Habing <sup>1</sup> ; <sup>1</sup> Dpt. of Veterinary Preventive Medicine, The Ohio State University, Columbus, OH, USA, <sup>2</sup> Dpt. of Animal Sciences, The Ohio State University, Columbus, OH, USA.
<b>014P</b>	Changes in the frequency of reported human illnesses associated with Salmonella subtypes recovered from Michigan dairy farms in either 2000-2001 or 2009.	<b>G. Habing<sup>1</sup></b> , S. Manning <sup>2</sup> , C. Bolin <sup>3</sup> , J.T. Rudrik <sup>4</sup> , J.B. Kaneene <sup>1</sup> ; <sup>1</sup> Michigan State University, East Lansing, MI, USA, <sup>2</sup> Microbiology and Molecular Genetics, Michigan State University, East Lansing, MI, USA, <sup>3</sup> Diagnostic Center for Population & Animal Health, Michigan State University, East Lansing, MI, USA, <sup>4</sup> Infectious Disease Division, Michigan Department of Community Health, Lansing, MI, USA.
<b>015P</b>	Distribution and characteristics of shipments of mail-order hatchlings containing outbreak-associated Salmonella subtypes	<b>G. Habing<sup>1</sup></b> , A. Haftman <sup>1</sup> , R. Krogwold <sup>2</sup> , L. Muñoz-Vargas <sup>1</sup> , C. Basler <sup>3</sup> ; <sup>1</sup> Veterinary Preventive Medicine, The Ohio State University, Columbus, OH, USA, <sup>2</sup> Veterinary Services, USDA, APHIS, Pickerington, OH, USA, <sup>3</sup> Centers for Disease Control and Prevention, Atlanta, GA, USA.
<b>016P</b>	Wild birds as Potential Vectors for Salmonella on Ohio Dairies	<b>A.E. Strait</b> ; The Ohio State University, Columbus, OH, USA.

(continued)

## ECOLOGY AND MANAGEMENT OF FOODBORNE AGENTS POSTERS

**Poster Session II - Monday 5:00 - 6:30 PM - Grand Ballroom Salon III - 7th floor**

**Section Leader: Yvette Johnson-Walker**

Poster assembly begins at noon Monday. Please remove your posters by 6:30 PM Monday.

Poster Presenters must be with their competition entry posters for possible judge interviews.

Name badge is required.

No.	Title	Authors
<b>017P</b>	Application of MALDI-TOF MS for the identification and characterization of AMR bacteria from wildlife associated with concentrated animal feeding operations	J.C. Chandler <sup>1</sup> , B. Wang <sup>1</sup> , J.E. Anders <sup>1</sup> , A.B. Franklin <sup>2</sup> , J.T. LeJeune <sup>3</sup> , J.E. Prenni <sup>4</sup> , J.C. Carlson <sup>2</sup> , <b>B. Bisha</b> <sup>1</sup> ; <sup>1</sup> Animal Science, University of Wyoming, Laramie, WY, USA, <sup>2</sup> National Wildlife Research Center, Department of Agriculture Animal and Plant Health Inspection Service, Fort Collins, CO, USA, <sup>3</sup> Food Animal Health Research Program, The Ohio State University, Wooster, OH, USA, <sup>4</sup> Proteomics and Metabolomics Facility, Colorado State University, Fort Collins, CO, USA.

## EPIDEMIOLOGY AND ANIMAL HEALTH ECONOMICS POSTERS

**Poster Session I - Sunday 6:30-8:00 PM - Grand Ballroom Salon III - 7th floor**

**Section Leader: Ashley Hill**

Poster assembly begins at 4 PM Sunday. Please remove your posters by 10:00 AM Monday.

Poster Presenters must be with their competition entry posters for possible judge interviews.

Name badge is required.

No.	Title	Authors
<b>018P</b>	Nasal, skin and tonsillar carriage of methicillin-resistant and zinc-resistant Staphylococcus aureus in piglets fed diets supplemented with zinc and chlortetracycline	<b>R.G. Amachawadi</b> <sup>1</sup> , H.M. Scott <sup>2</sup> , J. Vinasco <sup>2</sup> , J. Feldpausch <sup>3</sup> , M.D. Tokach <sup>3</sup> , S.S. Dritz <sup>1</sup> , J.L. Nelssen <sup>3</sup> , R.D. Goodband <sup>3</sup> , T.G. Nagaraja <sup>1</sup> ; <sup>1</sup> Diagnostic Medicine/Pathobiology, Kansas State University, Manhattan, KS, USA, <sup>2</sup> Veterinary Pathobiology, Texas A&M University, College Station, TX, USA, <sup>3</sup> Animal Sciences & Industry, Kansas State University, Manhattan, KS, USA.
<b>019P</b>	High lifetime and reproductive performance of sows in Southern EU commercial herds can be predicted by high numbers of pigs born alive at parity 1	<b>R. Iida</b> <sup>1</sup> , Y. Koketsu <sup>1</sup> , C. Piñeiro <sup>2</sup> ; <sup>1</sup> School of Agriculture, Meiji University, Kawasaki, Japan, <sup>2</sup> PigCHAMP Pro Europa S.L., Segovia, Spain.
<b>020P</b>	Abortion occurrence, repeatability and factors associated with abortions in female pigs in Southern EU commercial herds	<b>R. Iida</b> <sup>1</sup> , Y. Koketsu <sup>1</sup> , C. Piñeiro <sup>2</sup> ; <sup>1</sup> School of Agriculture, Meiji University, Kawasaki, Japan, <sup>2</sup> PigCHAMP Pro Europa S.L., Segovia, Spain.
<b>021P</b>	Interactions between climatic factors, parity and total pigs born for occurrences and numbers of stillborn piglets during hot and humid or cold seasons in breeding herds	<b>R. Iida</b> , Y. Koketsu; School of Agriculture, Meiji University, Kawasaki, Japan.

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**EPIDEMIOLOGY AND ANIMAL HEALTH ECONOMICS POSTERS**  
**Poster Session I - Sunday 6:30-8:00 PM - Grand Ballroom Salon III - 7th floor**

**Section Leader: Ashley Hill**

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Name badge is required.

No.	Title	Authors
022P	Mastitis and pregnancy loss in first lactation dairy cows	<b>M. Dahl</b> <sup>1</sup> , F. Maunsell <sup>1</sup> , K. Galvao <sup>1</sup> , A. De Vries <sup>2</sup> , C. Risco <sup>1</sup> , J. Hernandez <sup>1</sup> ; <sup>1</sup> College of Veterinary Medicine, University of Florida, Gainesville, FL, USA, <sup>2</sup> Animal Sciences, University of Florida, Gainesville, FL, USA
023P	Heat stress related dairy cow mortality during heat waves and control periods in rural Southern Ontario from 2010-2012	<b>K.E. Bishop-Williams</b> <sup>1</sup> , O. Berke <sup>1</sup> , D.L. Pearl <sup>1</sup> , K. Hand <sup>2</sup> , D.F. Kelton <sup>1</sup> ; <sup>1</sup> Department of Population Medicine, Ontario Veterinary College, Guelph, ON, Canada, <sup>2</sup> Strategic Solutions, Puslinch, ON, Canada
024P	HoBi-like pestiviruses persistently infected calves transmit the virus to calves, sheep, goats and pigs	<b>F.V. Bauermann</b> <sup>1</sup> , S.M. Falkenberg <sup>2</sup> , J.F. Ridpath <sup>1</sup> ; <sup>1</sup> Ruminant Disease and Immunology Unit, National Animal Disease Center, USDA, ARS, Ames, IA, USA, <sup>2</sup> Vaccine Development, Elanco Animal Health, Greenfield, IN, USA
025P	Canadian Integrated Program for Antimicrobial Resistance Surveillance(CIPARS) broiler chicken farm surveillance: 2013 Salmonella results	<b>S. Gow</b> <sup>1</sup> , A. Agunos <sup>2</sup> , D. Leger <sup>2</sup> , A. Deckert <sup>2</sup> ; <sup>1</sup> Public Health Agency of Canada, Saskatoon, SK, Canada, <sup>2</sup> Public Health Agency of Canada, Guelph, ON, Canada
026P	Prevalence of a highly virulent <i>Campylobacter jejuni</i> clone associated with sheep abortion in feedlot cattle in the United States	<b>Y. Tang</b> <sup>1</sup> , O. Sahin <sup>1</sup> , N. Pavlovic <sup>1</sup> , J. Lejeune <sup>2</sup> , J. Carlson <sup>3</sup> , Q. Zhang <sup>1</sup> ; <sup>1</sup> Veterinary Microbiology and Preventive Medicine, Iowa State University, Ames, IA, USA, <sup>2</sup> Food Animal Health Research Program, Ohio State University, Wooster, OH, USA, <sup>3</sup> National Wildlife Research Center, USDA APHIS, Fort Collins, CO, USA
027P	Modeling considerations in the analysis of associations between antimicrobial use and resistance in beef feedlot cattle	<b>N.R. Noyes</b> <sup>1</sup> , K.M. Benedict <sup>1</sup> , S.P. Gow <sup>2</sup> , C.L. Waldner <sup>3</sup> , R.J. Reid-Smith <sup>4</sup> , C.W. Booker <sup>5</sup> , S.J. Hannon <sup>5</sup> , T.A. McAllister <sup>6</sup> , P.S. Morley <sup>1</sup> ; <sup>1</sup> Clinical Sciences, Colorado State University, Fort Collins, CO, USA, <sup>2</sup> Laboratory for Foodborne Zoonoses, Public Health Agency of Canada, Saskatoon, SK, Canada, <sup>3</sup> Western College of Veterinary Medicine, University of Saskatchewan, Saskatoon, SK, Canada, <sup>4</sup> Laboratory of Foodborne Zoonoses, Public Health Agency of Canada, Guelph, ON, Canada, <sup>5</sup> Feedlot Health Management Services, Okotoks, AB, Canada, <sup>6</sup> Lethbridge Research Center, University of Lethbridge, Lethbridge, AB, Canada

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# **EPIDEMIOLOGY AND ANIMAL HEALTH ECONOMICS POSTERS**

**Poster Session I - Sunday 6:30-8:00 PM - Grand Ballroom Salon III - 7th floor**

**Section Leader: Ashley Hill**

Poster assembly begins at 4 PM Sunday. Please remove your posters by 10:00 AM Monday.

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Name badge is required.

No.	Title	Authors
<b>028P</b>	Molecular and statistical analysis of <i>Campylobacter</i> spp. carriage and antimicrobial resistance in mammalian wildlife and livestock species from Ontario farms (2010)	<b>M. Viswanathan</b> <sup>1</sup> , D.L. Pearl <sup>1</sup> , E.N. Taboada <sup>2</sup> , E.J. Parmley <sup>3</sup> , C.M. Jardine <sup>4</sup> ; <sup>1</sup> Population Medicine, University of Guelph, Guelph, ON, Canada, <sup>2</sup> Laboratory for Foodborne Zoonoses, Public Health Agency of Canada, Lethbridge, AB, Canada, <sup>3</sup> Laboratory for Foodborne Zoonoses, Public Health Agency of Canada, Guelph, ON, Canada, <sup>4</sup> Pathobiology, University of Guelph, Guelph, ON, Canada
<b>029P</b>	Population dynamics of multi-drug resistant <i>Salmonella</i> in feedlot cattle treated with ceftiofur or chlortetracycline	<b>N. Ohta</b> <sup>1</sup> , H.M. Scott <sup>1</sup> , S. Lawhon <sup>1</sup> , K. Norman <sup>1</sup> , J. Vinasco-Torres <sup>1</sup> , B. Norby <sup>2</sup> , G.H. Loneragan <sup>3</sup> ; <sup>1</sup> Veterinary Pathobiology, Texas A&M University, College Station, TX, USA, <sup>2</sup> Large Animal Clinical Sciences, Michigan State University, East Lansing, MI, USA, <sup>3</sup> Animal and Food Sciences, Texas Tech University, Lubbock, TX, USA
<b>030P</b>	Tracking association between wild life and avian influenza using GPS-CDMA based Telemetry System	<b>O.-K. Moon</b> <sup>1</sup> , Y.-M. Kang <sup>1</sup> , H. Yoon <sup>1</sup> , H. Lee <sup>2</sup> , W. Jeong <sup>1</sup> , J. Choi <sup>1</sup> , J.-Y. Park <sup>1</sup> , I.-K. Kim <sup>2</sup> , D.-H. Kim <sup>2</sup> , T.-H. Kang <sup>2</sup> , Y.-S. Kim <sup>1</sup> ; <sup>1</sup> Veterinary Epidemiology Division, Animal and Plant Quarantine Agency, Anyang, Korea, Republic of, <sup>2</sup> Korea Institute of Environmental Ecology, Daejeon, Korea, Republic of
<b>031P</b>	Analysis of the temporal features of highly pathogenic avian influenza (HPAI) H5N8 epidemic in South Korea, 2014	<b>J. Choi</b> , H. Yoon, O.-K. Moon, W. Jeong, Y.-M. Kang, Y.-S. Kim; Veterinary Epidemiology Division, Animal and Plant Quarantine Agency, Anyang, Korea, Republic of.
<b>032P</b>	A needs-based assessment of the drivers of lost productivity for camels of eastern Ethiopia.	<b>J.W. Coatney</b> <sup>1</sup> , P.J. Plummer <sup>1</sup> , M.J. Yaeger <sup>2</sup> , G. Mekonnen <sup>3</sup> , G. Tuli <sup>3</sup> , S. Gebre <sup>3</sup> ; <sup>1</sup> Veterinary Diagnostic and Production Animal Medicine, Iowa State University College of Veterinary Medicine, Ames, IA, USA, <sup>2</sup> Veterinary Pathology, Iowa State University College of Veterinary Medicine, Ames, IA, USA, <sup>3</sup> National Animal Health Disease Investigation Center, Sebeta, Ethiopia.
<b>033aP</b>	Examining the cross-species infectivity of human and swine specific torque teno viruses (TTVs)	K. Effertz, <b>S. Ramamoorthy</b> ; Veterinary and Microbiological Sciences, North Dakota State University, Fargo, ND, USA.

# IMMUNOLOGY POSTERS

**Poster Session II - Monday 5:00 - 6:30 PM - Grand Ballroom Salon III - 7th floor**

**Section Leaders: Laura C. Miller and Renukaradhya Gourapura**

Poster assembly begins at noon Monday. Please remove your posters by 6:30 PM Monday.

Poster Presenters must be with their competition entry posters for possible judge interviews.

Name badge is required.

No.	Title	Authors
<b>033P</b>	Swine toolkit progress for the US Veterinary Immune Reagent Network.	<b>J. Lunney</b> <sup>1</sup> , A. Crossman <sup>1</sup> , D. Chapa <sup>1</sup> , J. LaBresh <sup>2</sup> , L. Kakach <sup>2</sup> , Y. Sullivan <sup>2</sup> , B. Wagner <sup>3</sup> , A. Keggan <sup>3</sup> , S. Babasyan <sup>3</sup> , D. Tompkins <sup>4</sup> , E. Hudgens <sup>4</sup> , C. Baldwin <sup>4</sup> ; <sup>1</sup> USDA ARS BARC APDL, Beltsville, MD, USA, <sup>2</sup> Kingfisher Biotech, Inc., Saint Paul, MN, USA, <sup>3</sup> Cornell Univ., Ithaca, NY, USA, <sup>4</sup> Univ. of Massachusetts, Amherst, MA, USA.
<b>034P</b>	Induction of type-I interferons by a synthetic porcine reproductive and respiratory syndrome virus strain	<b>H. Sun</b> , H. Vu, A. Pattmaik, F. Osorio; School of Veterinary Medicine and Biomedical Sciences, University of Nebraska-Lincoln, Lincoln, NE, USA.
<b>035P</b>	Failure of CTB fused to Porcine arterivirus M and GP5 proteins to enhance the porcine reproductive and respiratory syndrome virus GP5-specific antibody response in pigs	E. Roques <sup>1</sup> , M. Lessard <sup>2</sup> , <b>D. Archambault</b> <sup>1</sup> ; <sup>1</sup> Biological Sciences, University of Quebec at Montreal, Montréal, QC, Canada, <sup>2</sup> Agriculture and Agri-Food Canada, Sherbrooke, QC, Canada.
<b>036P</b>	Advances in vaccine design: Developing a cross-conserved influenza vaccine for swine	<b>A.H. Gutierrez</b> <sup>1</sup> , C. Loving <sup>2</sup> , Z. Olson <sup>2</sup> , A. Vincent <sup>2</sup> , F. Terry <sup>3</sup> , L. Moise <sup>4</sup> , W. Martin <sup>3</sup> , A.S. De Groot <sup>4</sup> ; <sup>1</sup> Institute for Immunology and Informatics, University of Rhode Island, Providence, RI, USA, <sup>2</sup> Virus and Prion Diseases Research Unit, NADC, USDA ARS, Ames, IA, USA, <sup>3</sup> EpiVax, Inc., Providence, RI, USA, <sup>4</sup> Institute for Immunology and Informatics, University of Rhode Island & EpiVax, Inc., Providence, RI, USA.
<b>037P</b>	Performance assessment of a real-time polymerase chain reaction assay for porcine epidemic diarrhea virus to assess PEDV transmission in growing pigs	<b>L.C. Miller</b> , K. Crawford, K.M. Lager; VPDRU, USDA-ARS-NADC, Ames, IA, USA.
<b>038P</b>	Use of oral fluids for the detection of IgG, IgA, and IgM antibodies against porcine epidemic diarrhea virus (PEDV) from experimentally infected weaned pigs	<b>L. Bower</b> , L. Gimenez-Lirola, M. Bhandari, H. Hoang, D. Sun, D. Madson, D. Magstadt, P. Arruda, G. Stevenson, K.-J. Yoon; Veterinary Diagnostic and Production Animal Medicine, Iowa State University, Ames, IA, USA.
<b>039P</b>	Inhibition of Type I Interferon Induction by Nonstructural Proteins of Porcine Epidemic Diarrhea Virus.	<b>K. Shi</b> <sup>1,2</sup> , D. Yoo <sup>1</sup> ; <sup>1</sup> Department of Pathobiology, University of Illinois, Urbana-Champaign, IL, USA, <sup>2</sup> Guangxi Provincial Center for Animal Disease Control and Prevention, Nanning, China

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# IMMUNOLOGY POSTERS

**Poster Session II - Monday 5:00 - 6:30 PM - Grand Ballroom Salon III - 7th floor**

**Section Leaders: Laura C. Miller and Renukaradhya Gourapura**

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Poster Presenters must be with their competition entry posters for possible judge interviews.

Name badge is required.

No.	Title	Authors
<b>040P</b>	Safety and antibody response of pigs to an experimental Porcine Epidemic Diarrhea Virus (PEDV) Vaccine, Killed Virus	<b>D. Fredrickson</b> <sup>1</sup> , M. Bandrick <sup>1</sup> , L. Taylor <sup>1</sup> , D. Coleman <sup>2</sup> , T. Ricker <sup>1</sup> , A. Pfeiffer <sup>2</sup> , M. Huether <sup>2</sup> , J. Zhang <sup>1</sup> , R. Verhelle <sup>1</sup> , T. Hildebrand <sup>1</sup> , J. Hardham <sup>1</sup> , V. Rapp-Gabrielson <sup>1</sup> ; <sup>1</sup> Zoetis, Kalamazoo, MI, USA, <sup>2</sup> Zoetis, Lincoln, NE, USA
<b>041P</b>	Antigenic relationships among porcine epidemic diarrhea virus and transmissible gastroenteritis virus strains.	<b>C.-M. Lin</b> , X. Gao, T. Oka, A. Vlasova, M. Esseili, Q. Wang, L. Saif; Food Animal Health Research Program, OARDC, CFAES, Department of Veterinary Preventive Medicine, The Ohio State University, Wooster, OH, USA.
<b>042P</b>	Expression of Mycoplasma hyopneumoniae P97c recombinant protein in Escherichia coli and purification for putative adjuvant activity analysis	C. Karam <sup>1</sup> , É. Roques <sup>1</sup> , <b>C. Marchand</b> <sup>1</sup> , S. Bourgault <sup>2</sup> , D. Archambault <sup>1</sup> ; <sup>1</sup> Sciences Biologiques, Université du Québec à Montréal, Montréal, QC, Canada, <sup>2</sup> Département de Chimie, Université du Québec à Montréal, Montréal, QC, Canada
<b>043P</b>	Polyfunctional cytokine responses by central memory CD4+ T cells in response to bovine tuberculosis.	<b>M.F. Maggioli</b> <sup>1</sup> , M.V. Palmer <sup>2</sup> , H.M. Vordermeier <sup>3</sup> , A.O. Whelan <sup>3</sup> , W.R. Waters <sup>2</sup> ; <sup>1</sup> Iowa State University /National Animal Disease Center- Agricultural Research Service/ Infectious Bacterial Diseases of Livestock Research Unit, Ames, IA, USA, <sup>2</sup> Infectious Bacterial Diseases of Livestock Research Unit, National Animal Disease Center- Agricultural Research Service, Ames, IA, USA, <sup>3</sup> TB Research Group, Animal Health and Veterinary Laboratories Agency-Weybridge, New Haw, Addlestone, UK
<b>044P</b>	Adenosine triphosphate interactions with bovine purinoceptor 7	<b>H. Salzbrenner</b> , M. Orr, Y. Su, D. McClenahan; Biology, University of Northern Iowa, Cedar Falls, IA, USA.
<b>045P</b>	Quantification of oxylipid profiles in bovine mammary tissue and milk during <i>Streptococcus uberis</i> mastitis	<b>V.E. Ryman</b> , L.M. Sordillo; Large Animal Clinical Sciences, Michigan State University, East Lansing, MI, USA.
<b>046P</b>	Role of strategic vaccination of dairy cows during the non-lactating period on enhancing serum and milk antibody titers during early lactation	<b>O. Kerro Dego</b> , R.A. Almeida, S.I. Headrick, M.J. Lewis, G.M. Pighetti, S.P. Oliver; Animal Science, The University of Tennessee, Knoxville, TN, USA.
<b>047P</b>	Novel adjuvant enhances immune response to <i>Leptospira</i> bacterin	<b>J. Wilson-Welder</b> , D. Alt; Infectious Bacterial Disease of Livestock, National Animal Disease Center, ARS-USDA, Ames, IA, USA.

(continued)

## IMMUNOLOGY POSTERS

**Poster Session II - Monday 5:00 - 6:30 PM - Grand Ballroom Salon III - 7th floor**

**Section Leaders: Laura C. Miller and Renukaradhya Gourapura**

Poster assembly begins at noon Monday. Please remove your posters by 6:30 PM Monday.

Poster Presenters must be with their competition entry posters for possible judge interviews.

Name badge is required.

No.	Title	Authors
048P	Comparison of a novel point-of-care diagnostic, PCRun, with real time Leptospira PCR for detection of Leptospira antigen in canine samples	<b>B.E. Thiel</b> <sup>1</sup> , L.J. Larson <sup>1</sup> , O. Okwumabua <sup>2</sup> , R.D. Schultz <sup>1</sup> ; <sup>1</sup> Pathobiological Sciences, School of Veterinary Medicine University of Wisconsin-Madison, Madison, WI, USA, <sup>2</sup> Wisconsin Veterinary Diagnostic Laboratory; Pathobiological Sciences, School of Veterinary Medicine University of Wisconsin-Madison, Madison, WI, USA.
049P	Pre-clinical determination of therapeutic protein immunogenicity for companion animals	<b>F. Terry</b> <sup>1</sup> , A.H. Gutierrez <sup>2</sup> , G. Richard <sup>1</sup> , W. Martin <sup>1</sup> , A.S. De Groot <sup>1</sup> ; <sup>1</sup> EpiVax Inc., Providence, RI, USA, <sup>2</sup> Institute for Immunology and Informatics, University of Rhode Island, Providence, RI, USA.
050P	Highly accurate prediction of immunodominant B-cell epitopes of <i>Chlamydia</i> species using physicochemical and evolutionary properties of proteins	<b>K. Rahman</b> <sup>1</sup> , E. Chowdhury <sup>1</sup> , Y.-C. Juan <sup>1</sup> , K. Sachse <sup>2</sup> , B. Kaltenboeck <sup>1</sup> ; <sup>1</sup> Pathobiology, Auburn University, Auburn, AL, USA, <sup>2</sup> Federal Institute for Animal Health Jena Germany
051P	1 $\alpha$ ,25-Dihydroxyvitamin D3 inhibits differentiation and bone resorption of osteoclasts derived from Wistar rat bone marrow mononuclear cells	X. Liu, J. Bian, J. Gu, Y. Yuan, J. Li, <b>Z. Liu</b> ; College of Veterinary Medicine, Yangzhou University, Yangzhou, China.

# **PATHOBIOLOGY OF ENTERIC AND FOODBORNE PATHOGENS POSTERS**

**Poster Session I - Sunday 6:30-8:00 PM - Grand Ballroom Salon III - 7th floor**

**Section Leaders: Radhey S. Kaushik and Weiping Zhang**

Poster assembly begins at 4 PM Sunday. Please remove your posters by 10:00 AM Monday.

Poster Presenters must be with their competition entry posters for possible judge interviews.

Name badge is required.

No.	Title	Authors
<b>052P</b>	Comparative in vivo and in vitro studies of porcine rotavirus G9P[13] and human rotavirus Wa (G1P[8]) in gnotobiotic pigs.	<b>L. Shao</b> , L.J. Saif, D. Fischer, S. Kandasamy, A. Rauf, A. Vlasova; Veterinary Preventive Medicine, The Ohio State University, Wooster, OH, USA.
<b>053P</b>	Colonization dynamics and effect of human rotavirus infection on defined commensal microflora in a gnotobiotic (Gn) pig model	<b>H.-C. Huang</b> , A. Kumar, A.N. Vlasova, D. Fischer, S. Kandasamy, A. Rauf, L. Shao, L.J. Saif, G. Rajashekara; Food Animal Health Research Program, OARDC, The Ohio State University, Wooster, OH, USA.
<b>054P</b>	A fusion protein of <i>Escherichia coli</i> heat-labile toxoid (LT <sub>R192G</sub> ) and spike protein epitopes of the porcine epidemic diarrhea virus induced neutralizing antibodies against PEDV	<b>Y. Wang</b> , X. Ruan, R. Guo, Y. Fang, W. Zhang; Diagnostic Medicine/Pathobiology, Kansas State University College of Veterinary Medicine, Manhattan, KS, USA.
<b>055P</b>	Nucleocapsid protein of porcine epidemic diarrhea virus enhances viral replication <i>in vitro</i>	<b>R. Guo</b> , Y. Fang; Diagnostic Medicine/Pathobiology, College of Veterinary Medicine, Manhattan, KS, USA.
<b>056P</b>	Pilot study of comparative immunology and cross-protection of a US spike-insertion deletion (S-INDEL) strain of PEDV and original US PEDV strain.	<b>T. Annamalai</b> , C.-M. Lin, X. Liu, Z. Lu, H. Hu, X. Gao, L.J. Saif, Q. Wang; The Ohio State University, Wooster, OH, USA.
<b>057P</b>	Virulence of Porcine Epidemic Diarrhea virus (PEDV) for weaning-age pigs	<b>M. Bandrick</b> , D. Fredrickson, L. Taylor, J. Marx, T. Hildebrand, J. Johnson, A. Kaliyati, K. Segur, J. Hardham, V. Rapp-Gabrielson; VMRD, Zoetis, Kalamazoo, MI, USA.
<b>058P</b>	A comparison of serological tests for Lawsonia intracellularis in swine	R. Magtoto <sup>1</sup> , A. Vegi <sup>2</sup> , C. Wang <sup>1</sup> , J. Johnson <sup>1</sup> , <b>S. Ramamoorthy</b> <sup>2</sup> ; <sup>1</sup> Iowa State University, Ames, IA, USA, <sup>2</sup> Vet. Microbiological Sciences, N. Dakota State University, Fargo, ND, USA
<b>059P</b>	Comparative analysis of IS1096- and Himar1-derived transposon insertion sites in Mycobacterium avium subsp. paratuberculosis.	<b>G. Rathnaiah</b> <sup>1</sup> , J.P. Bannantine <sup>2</sup> , D.K. Zinniel <sup>1</sup> , J.R. Stabel <sup>2</sup> , Y.T. Grohn <sup>3</sup> , R.G. Barletta <sup>1</sup> ; <sup>1</sup> School of Veterinary Medicine and Biomedical Sciences, University of Nebraska, Lincoln, NE, USA, <sup>2</sup> National Animal Disease Center, Ames, IA, USA, <sup>3</sup> College of Veterinary Medicine, Cornell University, Ithaca, NY, USA
<b>060P</b>	Establishment and characterization of primary and immortalized bovine ileal epithelial cell lines from a young calf	P. Katwal <sup>1</sup> , T. Milton <sup>1</sup> , <b>R. Kaushik</b> <sup>2</sup> ; <sup>1</sup> Biology and Microbiology, South Dakota State University, Brookings, SD, USA, <sup>2</sup> Biology and Microbiology, and Veterinary and Biomedical Sci., South Dakota State University, Brookings, SD, USA.

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# PATHOBIOLOGY OF ENTERIC AND FOODBORNE PATHOGENS POSTERS

Poster Session I - Sunday 6:30-8:00 PM - Grand Ballroom Salon III - 7th floor

Section Leaders: Radhey S. Kaushik and Weiping Zhang

Poster assembly begins at 4 PM Sunday. Please remove your posters by 10:00 AM Monday.

Poster Presenters must be with their competition entry posters for possible judge interviews.

Name badge is required.

No.	Title	Authors
062P	Evaluation of conjugative transfer of antimicrobial resistance and virulence among multidrug resistant and pansusceptible <i>Salmonella</i> from the food-animal industry	<b>T.L. Poole</b> <sup>1</sup> , T.R. Callaway <sup>1</sup> , Y.-C. Hsieh <sup>2</sup> , T.J. Herrman <sup>2</sup> , T.S. Edrington <sup>1</sup> , D.M. Brichta-Harhay <sup>3</sup> , D.J. Nisbet <sup>1</sup> ; <sup>1</sup> Food and Feed Safety Research, USDA/ARS/SPARC, College Station, TX, USA, <sup>2</sup> AgriLife Research, Texas A&M University, College Station, TX, USA, <sup>3</sup> USDA/ARS/MARC, Clay Center, NE, USA
063P	Development and evaluation of DNA vaccines for <i>Campylobacter</i> control in poultry	<b>X. Liu</b> , X. Zeng, L. Jones, J. Lin; Department of Animal Science, University of Tennessee, Knoxville, TN, USA.
064P	Identification of the Factors Required for High Frequency Conjugation in <i>Campylobacter jejuni</i>	<b>X. Zeng</b> <sup>1</sup> , Z. Wu <sup>2</sup> , D. Ardeshta <sup>1</sup> , S. Brown <sup>1</sup> , B. Gillespie <sup>1</sup> , Q. Zhang <sup>2</sup> , J. Lin <sup>1</sup> ; <sup>1</sup> Animal Science, University of Tennessee, Knoxville, TN, USA, <sup>2</sup> Veterinary microbiology and preventive medicine, Iowa state university Ames, IA, USA
065P	Evaluation of passive immunotherapeutic efficacy of hyperimmunized egg-yolk powder against intestinal colonization of <i>campylobacter jejuni</i> in chickens	N.C. Paul, S. Al-Adwani, <b>D.H. Shah</b> ; Department of Veterinary Microbiology and Pathology, Washington State University, Pullman, WA, USA.
066P	Biofilm formation and antibiotic resistance among most prevalent poultry <i>Salmonella</i> serotypes isolated from US poultry	N.C. Paul <sup>1</sup> , R. Crespo <sup>1</sup> , J. Guard <sup>2</sup> , <b>D.H. Shah</b> <sup>1</sup> ; <sup>1</sup> Department of Veterinary Microbiology and Pathology, Washington State University, Pullman, WA, USA, <sup>2</sup> Egg Quality and Safety Research Unit, ARS, United States Department of Agriculture, Athens, GA, USA

## RESPIRATORY DISEASES POSTERS

**Poster Session II - Monday 5:00 - 6:30 PM - Grand Ballroom Salon III - 7th floor**

**Section Leaders: Amelia R. Woolums and Chris Chase**

Poster assembly begins at noon Monday. Please remove your posters by 6:30 PM Monday.

Poster Presenters must be with their competition entry posters for possible judge interviews.

Name badge is required.

No.	Title	Authors
067P	Diagnostic investigation of an outbreak of respiratory and reproductive disorder in a Polish pig farm.	M. Rajska <sup>1</sup> , K. Biernacka <sup>2</sup> , R. Rauh <sup>3</sup> , <b>T. Stadejek</b> <sup>2</sup> ; <sup>1</sup> Vet-com, Olsztyn, Poland, <sup>2</sup> Faculty of Veterinary Medicine, Warsaw University of Life Sciences, Warsaw, Poland, <sup>3</sup> Tetracore Inc., Rockville, MD, USA
068P	Simultaneous detection of PRRSV and SIV by a one-step triplex real time RT-PCR assay	<b>Xiju Shi</b> <sup>1,3</sup> , Q. Sun <sup>2</sup> , A. Beckley <sup>1</sup> , J. Shi <sup>1</sup> , J. Bai <sup>2</sup> ; <sup>1</sup> Department of Anatomy & Physiology, College of Veterinary Medicine Kansas State University, Manhattan, KS, USA, <sup>2</sup> Kansas State Veterinary Diagnostic Laboratory, College of Veterinary Medicine Kansas State University, Manhattan, KS, USA, <sup>3</sup> Beijing Entry-Exit Inspection and Quarantine Bureau, Beijing, China
069P	Comparative analysis of routes of immunization of a live PRRS virus vaccine in a heterologous virus challenge study	<b>K. Ouyang</b> , J. Hiremath, B. Binjawadagi, G.J. Renukaradhya; Food Animal Health Research Program, OARDC, The Ohio State University, Wooster, OH, USA.
070P	Quantification of UV-induced RNA damage of porcine respiratory and reproductive syndrome virus	<b>B. Park</b> , L. Jae Woo, Y. Jun Seok, H. Jeong Hee; College of Veterinary Medicine, Kangwon National University, Chuncheon, Korea, Republic of.
071P	Effect of bovine viral diarrhea virus (BVDV) infection on neutrophil survival and surface marker expression	<b>N. Thakur</b> , L. Braun, C.C.L. Chase; Animal Disease Research & Diagnostic Lab., South Dakota State University, Brookings, SD, USA.
072P	Development of automated DNA microarray based assays for multiplex detection of bovine pathogens	<b>A. Ambagala</b> <sup>1</sup> , N. Thantrige-Don <sup>2</sup> , T. Furukawa-Stoffer <sup>1</sup> , C. Buchanan <sup>1</sup> , T. Joseph <sup>3</sup> , D. Godson <sup>4</sup> , J. Gilleard <sup>5</sup> , T. Alexander <sup>6</sup> , O. Lung <sup>1</sup> ; <sup>1</sup> National Centres for Animal Disease, Canadian Food Inspection Agency, Lethbridge, AB, Canada, <sup>2</sup> Faculty of Veterinary Medicine, University of Calgary, Calgary, AB, Canada, <sup>3</sup> Animal Health Centre, Ministry of Agriculture, Abbotsford, BC, Canada, <sup>4</sup> Prairie Diagnostic Services Inc, Saskatoon, SK, Canada, <sup>5</sup> Faculty of Veterinary Medicine, University of Calgary, Calgary, AB, Canada, <sup>6</sup> Lethbridge Research Centre, Agriculture and Agri-Food Canada, Lethbridge, AB, Canada.
073P	Efficacy test of broad spectrum Canine Influenza Vaccine in guinea pigs	<b>M. Yeom</b> <sup>1</sup> , D. Song <sup>1</sup> , W. Na <sup>1</sup> , M. Hong <sup>1</sup> , D.-G. Jung <sup>1</sup> , J.-K. Kim <sup>2</sup> ; <sup>1</sup> KRIBB, Daejeon, Korea, Republic of, <sup>2</sup> Korea University, Sejong, Korea, Republic of

## VECTOR-BORNE AND PARASITIC DISEASES POSTERS

**Poster Session II - Monday 5:00 - 6:30 PM - Grand Ballroom Salon III - 7th floor**

**Section Leader: Roman Ganta and Roger W. (Bill) Stich**

Poster assembly begins at noon Monday. Please remove your posters by 6:30 PM Monday.

Poster Presenters must be with their competition entry posters for possible judge interviews.

Name badge is required.

No.	Title	Authors
<b>074P</b>	Investigating white-tailed deer as a potential amplifying host for <i>Borrelia miyamotoi</i> , a relapsing fever group spirochete transmitted by blacklegged ticks.	<b>S. Han<sup>1</sup></b> , J.I. Tsao <sup>2</sup> ; <sup>1</sup> Comparative Medicine and Integrative Biology, Michigan State University, East Lansing, MI, USA, <sup>2</sup> Fisheries and Wildlife, Large Animal Clinical Science, Michigan State University, East Lansing, MI, USA.

## VIRAL PATHOGENESIS POSTERS

**Poster Session II - Monday 5:00 - 6:30 PM - Grand Ballroom Salon III - 7th floor**

**Section Leader: Kyoung-Jin Yoon**

Poster assembly begins at noon Monday. Please remove your posters by 6:30 PM Monday.

Poster Presenters must be with their competition entry posters for possible judge interviews.

Name badge is required.

No.	Title	Authors
<b>075P</b>	Assessing the role of PRRSV envelope minor glycoprotein 3 in the induction of an immune response in swine using a heterologous prime-boost vaccination schedule	<b>K. Kimpston-Burkgren<sup>1</sup></b> , H. Vu <sup>2</sup> , B. Kwon <sup>2</sup> , A. Pattnaik <sup>2</sup> , F. Osorio <sup>2</sup> ; <sup>1</sup> School of Biological Sciences, University of Nebraska-Lincoln, Lincoln, NE, USA, <sup>2</sup> School of Veterinary Medicine and Biomedical Sciences, University of Nebraska-Lincoln, Lincoln, NE, USA.
<b>076P</b>	Mutations in the highly conserved GKYLQRRLQ motif of nsp1 $\beta$ protein impairs the innate immune suppression function of porcine reproductive and respiratory syndrome virus (PRRSV)	<b>Y. Li</b> , P. Shang, Y. Fang; Diagnostic Medicine / Pathobiology, Kansas State university, Manhattan, KS, USA.
<b>077P</b>	Attenuation of porcine reproductive and respiratory syndrome virus (PRRSV) by inactivating the expression of ribosomal frameshifting products nsp2TF and nsp2N	<b>Y. Li<sup>1</sup></b> , L. zhu <sup>1</sup> , R. Ransburg <sup>1</sup> , A.E. Firth <sup>2</sup> , E.J. Snijder <sup>3</sup> , Y. Fang <sup>1</sup> ; <sup>1</sup> Diagnostic Medicine / Pathobiology, Kansas State university, Manhattan, KS, USA, <sup>2</sup> Department of Pathology, University of Cambridge, Cambridge, UK, <sup>3</sup> Department of Medical Microbiology, Leiden university Medical center, Leiden, Netherlands.
<b>078P</b>	In vitro inhibition of porcine reproductive and respiratory syndrome virus (PRRSV) replication by specific DNA aptamers	<b>C.A. Gagnon</b> , C. Savard, C. Provost; Faculté de médecine Vétérinaire, Université de montréal, Saint-Hyacinthe, QC, Canada.
<b>079P</b>	Induction mechanisms of type-I IFN, inflammation, and apoptosis by H3N2 canine influenza virus in canine tracheal epithelial cells	<b>W.J. Park</b> , B.J. Park, H.S. Ahn, J.B. Lee, S.Y. Park, C.S. Song, S.W. Lee, I.S. Choi; Department of Infectious Diseases, College of Veterinary Medicine, Konkuk University, Seoul, Korea, Republic of.

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# VIRAL PATHOGENESIS POSTERS

**Poster Session II - Monday 5:00 - 6:30 PM - Grand Ballroom Salon III - 7th floor**

**Section Leader: Kyoung-Jin Yoon**

Poster assembly begins at noon Monday. Please remove your posters by 6:30 PM Monday.

Poster Presenters must be with their competition entry posters for possible judge interviews.

Name badge is required.

No.	Title	Authors
080P	Serologic surveillance of antibody against seasonal human flu (H1N1 & H3N2) and canine flu (H3N8) viruses in dogs in Ohio	H. Jang <sup>1</sup> , Y. Jackson <sup>2</sup> , J. B. Daniels <sup>3</sup> , A. Ali <sup>4</sup> , K.-I. Kang <sup>1</sup> , M. Elaish <sup>1</sup> , C.-W. Lee <sup>1</sup> ; <sup>1</sup> Food Animal Health Research Program, The Ohio State University, Wooster, OH, USA, <sup>2</sup> Department of Animal Sciences, The Ohio State University, Columbus, OH, USA, <sup>3</sup> Department of Veterinary Clinical Science, College of Veterinary Medicine, The Ohio State University, Columbus, OH 43210, USA, <sup>4</sup> Department of Poultry Diseases, Beni-Suef University, Beni-Suef, Egypt.
081P	Measurement of tissue factor activity as a biomarker of infection of the spinal cord vasculature with equine herpesvirus-1	<b>C.L. Holz</b> , J.P. Luyendyk, A.K. Kopec, R.K. Nelli, S.B. Hussey, J. Dau, G. Soboll Hussey; Pathobiology and Diagnostic Investigation, Michigan State University, East Lansing, MI, USA.
082P	Sensitivity improvement of pan-viral DNA array and high-throughput sequencing with propidium monoazide (PMA) for the identification of viruses from tissue samples	<b>C.A. Gagnon</b> <sup>1</sup> , C. Bellehumeur <sup>1</sup> , B. Boyle <sup>2</sup> , J. Harel <sup>1</sup> , S. Charette <sup>2</sup> , Y. L'Homme <sup>3</sup> , L. Masson <sup>4</sup> ; <sup>1</sup> Faculté de Médecine Vétérinaire, Université de Montréal, St-Hyacinthe, QC, Canada, <sup>2</sup> Institut de biologie intégrative et des systèmes, Université Laval, Québec, QC, Canada, <sup>3</sup> Saint-Hyacinthe Laboratory, Canadian food inspection agency, St-Hyacinthe, QC, Canada, <sup>4</sup> Montréal, National Research Council Canada, Montréal, QC, Canada.
083P	Development of monoclonal antibodies and other reagents for detection of porcine deltacoronavirus (PDCoV)	A. Singrey, S. Lawson, F. Okda, K. Hain, T. Clement, J. Christopher-Hennings, <b>E.A. Nelson</b> ; Veterinary & Biomedical Sciences, South Dakota State University, Brookings, SD, USA.
084P	Evaluation of oral fluids as diagnostic specimens detecting porcine epidemic diarrhea virus (PEDV) shedding in experimentally infected weaned pigs	<b>L. Bower</b> , M. Bhandari, D. Madson, H. Hoang, D. Magstadt, P. Arruda, D. Sun, B.L. Wilberts, G. Stevenson, K.-J. Yoon; Veterinary Diagnostic and Production Animal Medicine, Iowa State University, Ames, IA, USA.
085P	Quantification of uv-exposed porcine epidemic diarrhea virus using real-time rt-pcr	<b>J. Jo</b> , B. Park, J. Yi, J. Han; College of Veterinary Medicine, Kangwon National University, Chuncheon, Korea, Republic of.
086P	The induction of apoptosis in infected monocytes by cytopathic bovine viral diarrhea virus	G. Seong, <b>K.-S. Choi</b> ; Animal Science, Kyungpook National University, Sangju, Korea, Republic of.
087P	Assessment of ultraviolet irradiation-induced RNA damage on classical swine fever virus by realtime RT-PCR	<b>J. Yi</b> , B. Park, Y. Kim, J. Lim, Y. Lee, J. Han; College of Veterinary Medicine, Kangwon National University, Chuncheon, Korea, Republic of.

# **VIRAL PATHOGENESIS POSTERS**

**Poster Session II - Monday 5:00 - 6:30 PM - Grand Ballroom Salon III - 7th floor**

**Section Leader: Kyoung-Jin Yoon**

Poster assembly begins at noon Monday. Please remove your posters by 6:30 PM Monday.

Poster Presenters must be with their competition entry posters for possible judge interviews.

Name badge is required.

<b>No.</b>	<b>Title</b>	<b>Authors</b>
<b>088P</b>	Infection of post-weaning gnotobiotic pigs with genogroup II human norovirus.	<b>B.J. Park</b> , W.J. Park, J.B. Lee, S.Y. Park, C.S. Song, S.W. Lee, I.S. Choi*; Department of Infectious Disease, College of Veterinary Medicine, Konkuk university, Seoul, Korea, Republic of.
<b>089P</b>	Detection of hepatitis E virus in rabbits in Korea.	<b>H.S. Ahn</b> , B.J. Park, W.J. Park, J.B. Lee, S.Y. Park, C.S. Song, S.W. Lee, I.S. Choi*; College of Veterinary Medicine, Konkuk University, Seoul, Korea, Republic of.

# **ORAL PROGRAM**

**BACTERIAL PATHOGENESIS**  
**Avenue Ballroom - 4<sup>th</sup> Floor**  
**Section Leader: Gireesh Rajashekara**

Time	No.	Title	Authors
<b>8:00 Mon.</b>	<b>001</b>	Identification of a Type II Toxin-antitoxin System in <i>Campylobacter jejuni</i>	<b>Z. Shen</b> , R. Patil, O. Sahin, Q. Zhang; Iowa State University, Ames, IA, USA.
<b>8:15</b>	<b>002</b>	A single nucleotide change in <i>mutY</i> leads to the increased emergence of fluoroquinolones resistant mutants in <i>Campylobacter jejuni</i>	<b>L. Dai</b> , W. Muraoka, Z. Wu, Q. Zhang; Iowa State University, Ames, IA, USA.
<b>8:30</b>	<b>003</b>	Transcriptomic responses of a <i>Campylobacter jejuni</i> strain associated with sheep abortion to sheep whole blood and identification of a novel abortion-inducing factor	<b>Z. Wu</b> <sup>1</sup> , D. Kurkiewicz <sup>2</sup> , O. Sahin <sup>1</sup> , M. Yaeger <sup>3</sup> , P. Liu <sup>2</sup> , P. Plummer <sup>4</sup> , Z. Shen <sup>1</sup> , Q. Zhang <sup>1</sup> ; <sup>1</sup> Department of Veterinary Microbiology and Preventive Medicine, Iowa State University, Ames, IA, USA, <sup>2</sup> Department of Statistics, Iowa State University, Ames, IA, USA, <sup>3</sup> Department of Veterinary Pathology, Iowa State University, Ames, IA, USA, <sup>4</sup> Department of Vet Diagnostic and Production Animal Medicine, Iowa State University, Ames, IA, USA.
<b>8:45</b>	<b>004</b>	Environmental persistence and biofilm formation of hypermucoid and non-hypermucoid <i>Klebsiella pneumoniae</i>	<b>E. Soto</b> , A. Loftis, S. Rostad, A. Beierschmitt, I. Halliday-Simmons; Biomedical Sciences, Ross University-School of Veterinary Medicine, Basseterre, Saint Kitts and Nevis.
<b>9:00</b>	<b>005</b>	Characterization of Glucosamine-6-Phosphate Synthase (GlmS) mutant of <i>Salmonella</i> : Effect on Biology and Pathogenesis of the Organism	A. Bennett, D. Shippy, <b>A.A. Fadi</b> ; Animal Sciences, University of Wisconsin-Madison, Madison, WI, USA.
<b>9:15 Mon.</b>	<b>006</b>	Human specific virulence factors absent in LA-MRSA ST5 strains isolated from pigs, swine facilities, and humans with swine contact.	<b>S.J. Hau</b> <sup>1</sup> , T. Frana <sup>1</sup> , P.R. Davies <sup>2</sup> , J. Sun <sup>2</sup> , T.L. Nicholson <sup>3</sup> ; <sup>1</sup> Department of Veterinary Diagnostic and Production Animal Medicine, College of Veterinary Medicine, Iowa State University, Ames, IA, USA, <sup>2</sup> Department of Veterinary Population Medicine, College of Veterinary Medicine, University of Minnesota, St. Paul, MN, USA, <sup>3</sup> National Animal Disease Center, Agricultural Research Service, USDA, Ames, IA, USA.
<b>9:30</b>		<b>Break and Table Top Exhibits – Foyer</b>	
<b>10:00</b>	<b>007</b>	Global control of virulence and survival of <i>Mycobacterium avium</i> subsp. <i>paratuberculosis</i> .	<b>A.M. Talaat</b> , P. Ghosh; University of Wisconsin-Madison, Madison, WI, USA.
<b>10:15 Mon.</b>	<b>008</b>	Overexpression of catalytically inactive dimethyl adenosine transferase (KsgA) unveils contribution of KsgA to <i>Salmonella</i> Enteritidis physiology and virulence	<b>K. Chiok</b> , D.H. Shah; Department of Veterinary Microbiology and Pathology, Washington State University, Pullman, WA, USA.
<b>10:30</b>		Open	

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**BACTERIAL PATHOGENESIS**  
**Avenue Ballroom - 4<sup>th</sup> Floor**  
**Section Leader: Gireesh Rajashekara**

<b>Time</b>	<b>No.</b>	<b>Title</b>	<b>Authors</b>
<b>10:45-11:30 Mon.</b> Keynote	<b>009</b>	<b>Bacterial Pathogenesis Keynote:</b> Towards an understanding of Salmonella persistence in pigs.	<b>R. Isaacson</b> ; Veterinary and Biomedical Sciences, University of Minnesota, St. Paul, MN, USA.
<b>11:30</b>		<b>Lunch Break</b>	
<b>Time</b>	<b>No.</b>	<b>Title</b>	<b>Authors</b>
		<b>ACVM-CE Mini-Symposium</b>	
<b>1:30-2:00 Mon.</b>	<b>010</b>	Brucellosis in animal reservoirs in the united states: challenges and opportunities	<b>S. Olsen</b> ; National Animal Disease Center, Ames, IA, USA.
<b>2:00-2:30</b>	<b>011</b>	Bovine tuberculosis eradication: Update and emerging issues	<b>M. Palmer</b> ; Infectious Bacterial Diseases of Livestock, National Animal Disease Center, USDA, Ames, IA, USA.
<b>2:30</b>		Open	
<b>2:45</b>		<b>Break and Table Top Exhibits – Foyer</b>	
<b>3:00-3:30</b>	<b>012</b>	Arbovirus research and epizootic hemorrhagic disease	<b>D.S. McVey</b> , M.G. Ruder; ABADRU, USDA ARS, Manahattan, KS, USA.
<b>3:30-4:00 Mon.</b>	<b>013</b>	Has changing animal husbandry practices changed the ecology of influenza?	<b>K.M. Lager</b> ; National Animal Disease Center, USDA-ARSNADC, Ames, IA, USA.
<b>4:00</b>		Open	
<b>4:15</b>		Open	
<b>4:30 to 5:00</b>	<b>5:00</b>	<b>Break and Table Top Exhibits – Foyer</b>	
<b>5:00 to 6:30</b>		<b>Poster Session II Grand Ballroom Salon III - 7th floor</b>	



# BACTERIAL PATHOGENESIS

Avenue Ballroom - 4<sup>th</sup> Floor

Section Leader: Gireesh Rajashekara

Time	No.	Title	Authors
8:00 Tues.	014	Mannheimia haemolytica biofilm formation on bovine respiratory epithelial cells	<b>I. Boukahil</b> , C.J. Czuprynski; University of Wisconsin-Madison School of Veterinary Medicine, Madison, WI, USA.
8:15	015	Characterization of a novel isolate of <i>Leptospira</i> associated with the greater white-toothed shrew ( <i>Crocidura russula</i> ), an invasive mammalian species in Ireland.	<b>J.E. Nally</b> <sup>1</sup> , Z. Arent <sup>2</sup> , M.V. Palmer <sup>1</sup> , C. Gilmore <sup>2</sup> , A. McDevitt <sup>3</sup> , S. Regan <sup>3</sup> , J. Yearsley <sup>3</sup> , B.J. McMahon <sup>3</sup> ; <sup>1</sup> ARS, National Animal Disease Center, Ames, IA, USA, <sup>2</sup> AFBI, Belfast, UK, <sup>3</sup> UCD, Dublin, Ireland
8:30	016	Complete genome sequencing and analysis of the red blood cell pathogen of llamas and alpacas, <i>Candidatus Mycoplasma haemolamae</i> □	<b>A.M.S. Guimaraes</b> <sup>1</sup> , B. Toth <sup>2</sup> , A.P. Santos <sup>2</sup> , N.C. do Nascimento <sup>2</sup> , J.E. Kritchevsky <sup>2</sup> , J.B. Messick <sup>2</sup> ; <sup>1</sup> University of São Paulo, São Paulo, Brazil, <sup>2</sup> Purdue University, West Lafayette, IN, USA
8:45	017	Insulated isothermal PCR assay for the detection of <i>Taylorella equigenitalis</i>	Y. tsai <sup>1</sup> , S. Artiushin <sup>2</sup> , U. Balasuriya <sup>2</sup> , H. Chang <sup>1</sup> , C. tsai <sup>1</sup> , L. Ma <sup>1</sup> , P.A. Lee <sup>1</sup> , H.G. Chang <sup>1</sup> , <b>H.T. Wang</b> <sup>1</sup> ; <sup>1</sup> GeneReach, TAICHUNG, Taiwan, <sup>2</sup> Maxwell H. Gluck Equine Research Center, Lexington, KY, USA.
9:00	018	An internal control improves the detection of <i>Brucella canis</i> in diagnostic samples analyzed by a triplex real-time PCR assay	<b>J. Bai</b> , E. Schirtzinger, B. An, G. Andereson; Kansas State Veterinary Diagnostic Laboratory, Kansas State University, Manhattan, KS, USA.
9:15 Tues.	019	<i>Actinobacillus pleuropneumoniae</i> (APP) ApxIV toxin antibody ontogeny in serum, oral fluid and fecal specimens from animals inoculated under experimental conditions.	<b>W. Gonzalez</b> <sup>1</sup> , L.G. Giménez-Lirola <sup>1</sup> , M. Gottschalk <sup>2</sup> , C. Wang <sup>1</sup> , A. Holmes <sup>1</sup> , S. Lizano <sup>3</sup> , C. Goodell <sup>3</sup> , K. Poonsunk <sup>1</sup> , P. Sitthichaoenchai <sup>1</sup> , J. Zimmerman <sup>1</sup> ; <sup>1</sup> Iowa State University, Ames, IA, USA, <sup>2</sup> Faculté de Médecine Vétérinaire, Université de Montréal, St. Hyacinthe, QC, Canada, <sup>3</sup> IDEXX Laboratories, Inc, Westbrook, MD, USA
9:30		Break	
11:45 to	12:30	<b>Business Meeting, Dedication, New Members Introduction, and Graduate Student Competition Awards Presentations</b>	

**BIOSAFETY AND BIOSECURITY**  
**Denver/Houston Room - 5th Floor**  
**Section Leader: Gabriele Landolt**

<b>Time</b>	<b>No.</b>	<b>Title</b>	<b>AuthorBlock</b>
<b>1:30 Mon.</b>	<b>021</b>	Prevalence, genotypes, and risk factors for <i>Clostridium perfringens</i> among Ontario broiler chicken flocks	<b>H. Kasab-Bachi</b> , S. McEwen, D. Pearl, D. Slavic, M. Guerin; Population Medicine, University of Guelph, Guelph, ON, Canada.
<b>1:45</b>	<b>022</b>	Seroprevalence and risk factors for <i>Coxiella burnetii</i> exposure in Ontario sheep and goats	S. Meadows <sup>1</sup> , <b>A. Jones-Bitton</b> <sup>1</sup> , J.T. Jansen <sup>2</sup> , S.A. McEwen <sup>1</sup> , P.I. Menzies <sup>1</sup> ; <sup>1</sup> Department of Population Medicine, University of Guelph, Guelph, ON, Canada, <sup>2</sup> Veterinary Science and Policy, Ontario Ministry of Agriculture, Food and Rural Affairs, Elora, ON, Canada.
<b>2:00</b>	<b>023</b>	Identifying cattle farms and areas for risk-based surveillance for bovine tuberculosis in Minnesota	J. Ribeiro Lima <sup>1</sup> , S. Schwabenlander <sup>2</sup> , M. Oakes <sup>3</sup> , B. Thompson <sup>2</sup> , <b>S.J. Wells</b> <sup>1</sup> ; <sup>1</sup> Veterinary Population Medicine, School of Veterinary Medicine, University of Minnesota, St. Paul, MN, USA, <sup>2</sup> Minnesota Board of Animal Health, St. Paul, MN, USA, <sup>3</sup> Epidemiology and Community Health, School of Public Health, University of Minnesota, St. Paul, MN, USA.
<b>2:15</b>	<b>024</b>	Detection of <i>Salmonella enterica</i> in the dairy environment using a commercially available lateral flow immunoassay.	<b>E. Doster</b> <sup>1</sup> , B. Burgess <sup>2</sup> , J. Elam <sup>3</sup> , K. Pabilonia <sup>1</sup> , N. Slovis <sup>3</sup> , P. Morley <sup>1</sup> ; <sup>1</sup> Clinical Sciences, Colorado State University, Fort Collins, CO, USA, <sup>2</sup> Population Health Sciences, Virginia Tech, Blacksburg, VA, USA, <sup>3</sup> Hagyard Equine Medical Institute, Lexington, KY, USA.
<b>2:30</b>	<b>025</b>	Improved characterization of <i>Salmonella enterica</i> shedding among reptile patients at the James L. Voss Veterinary Teaching Hospital	<b>A.C. Fagre</b> ; Department of Clinical Sciences, Colorado State University College of Veterinary Medicine and Biomedical Sciences, Fort Collins, CO, USA.
<b>2:45</b>		<b>Break and Table Top Exhibits – Foyer</b>	
<b>3:00-3:45 Mon. Keynote</b>	<b>026</b>	<b>Biosafety and Biosecurity Keynote:</b> Biosecurity, a biography: ten years of hard lessons.	<b>H. Aceto</b> ; Clinical Studies - New Bolton Center, University of Pennsylvania, School of Veterinary Medicine, Kennett Square, PA, USA.
<b>3:45</b>	<b>027</b>	Evaluation of POKIT, field deployable technology, for molecular based detection of Foot and Mouth Disease virus, Classical Swine Fever virus and African Swine Fever virus.	<b>J.D. Trujillo</b> <sup>1</sup> , Y.-L. Tsai <sup>2</sup> , Y.-C. Ln <sup>2</sup> , H.-H. Chang <sup>2</sup> , B.-H. Chen <sup>2</sup> , Y.-H. Shen <sup>2</sup> , P.-Y. Lee <sup>2</sup> , H.-F. Chang <sup>2</sup> , T. Wang <sup>2</sup> , I. .Morozov <sup>3</sup> ; <sup>1</sup> Iowa State University, Ames, IA, USA, <sup>2</sup> GeneReach, USA, MA, USA, <sup>3</sup> DHS Center of Excellence for Emerging and Zoonotic Animal Diseases, Kansas State University, Manhattan, KS, USA.
<b>4:00 Mon.</b>	<b>028</b>	Using social network analysis to understand epidemic potential in equine populations: a pilot study.	<b>K.L. Spence</b> , B. Goh, T. O'Sullivan, A.L. Greer; Department of Population Medicine, Ontario Veterinary College, University of Guelph, Guelph, ON, Canada.

**BIOSAFETY AND BIOSECURITY**  
**Denver/Houston Room - 5th Floor**  
**Section Leader: Gabriele Landolt**

Time	No.	Title	AuthorBlock
<b>4:15 Mon.</b>	<b>029</b>	Assessing spatial dispersal of pathogens around human settlements	<b>J.W. Yoo</b> <sup>1</sup> , R.B. Garabed <sup>1</sup> , J. Lee <sup>2</sup> , M. Moritz <sup>3</sup> , T.K. Manchang <sup>4</sup> , A. Drent <sup>1</sup> ; <sup>1</sup> College of Veterinary Medicine, Ohio State University, Columbus, OH, USA, <sup>2</sup> College of Public Health, Ohio State University, Columbus, OH, USA, <sup>3</sup> College of Arts and Sciences, Ohio State University, Columbus, OH, USA, <sup>4</sup> IRAD, Wakwa, Cameroon
<b>4:30</b>	<b>030</b>	Survey of Iowa residential bats for influenza viruses	<b>S. Azeem</b> , K. O'Neill, S. Kostohryz, A. Allam, D. Sun, L. Bower, K. Schwartz, K. J. Yoon; College of Veterinary Medicine, Iowa State Univ., Ames, IA, USA.
<b>4:45 to 5:00</b>	<b>5:00</b>	<b>Break and Table Top Exhibits – Foyer</b>	
<b>5:00 to</b>	<b>6:30</b>	<b>Poster Session II Grand Ballroom Salon III - 7th floor</b>	

**COMPANION ANIMAL EPIDEMIOLOGY**  
**Michigan/Michigan State Room - 6th Floor**  
**Section Leader: Audrey Ruple-Czerniak and Laura Hungerford**

Time	No.	Title	AuthorBlock
		<b>Presiders: Audrey Ruple-Czerniak and Nadia Saklou</b>	
<b>8:00 Mon.</b>	<b>031</b>	Scrotal castration as a safe and effective means of male canine sterilization	<b>K.A. Woodruff</b> <sup>1</sup> , K. Rigdon-Brestle <sup>2</sup> , P.A. Bushby <sup>1</sup> , C.L. Huston <sup>3</sup> , R.W. Wills <sup>3</sup> ; <sup>1</sup> Clinical Sciences, Mississippi State University College of Veterinary Medicine, Mississippi State, MS, USA, <sup>2</sup> Humane Alliance, Ashville, NC, USA, <sup>3</sup> Pathobiology and Population Medicine, Mississippi State University College of Veterinary Medicine, Mississippi State, MS, USA
<b>8:15</b>	<b>032</b>	Factors associated with hematuric struvite crystalluria in cats evaluated at general care veterinary hospitals in the United States (2007-2011)	<b>C.C. Okafor</b> <sup>1</sup> , D.L. Pearl <sup>1</sup> , S.L. Blois <sup>2</sup> , S.L. Lefebvre <sup>3</sup> , M. Yang <sup>3</sup> , M. Wang <sup>3</sup> , E.M. Lund <sup>3</sup> , C.E. Dewey <sup>1</sup> ; <sup>1</sup> Population Medicine, University of Guelph, Guelph, ON, Canada, <sup>2</sup> Clinical Studies, University of Guelph, Guelph, ON, Canada, <sup>3</sup> Banfield Pet Hospital, Portland, OR, USA

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**COMPANION ANIMAL EPIDEMIOLOGY**  
**Michigan/Michigan State Room - 6th Floor**

**Section Leader: Audrey Ruple-Czerniak and Laura Hungerford**

<b>Time</b>	<b>No.</b>	<b>Title</b>	<b>AuthorBlock</b>
<b>8:30 Mon.</b>	<b>033</b>	Prevalence and association with Feline Upper Respiratory Disease severity for the detection of select pathogens and risk factors in Midwestern animal shelter cats	<b>U. Donnett<sup>1</sup></b> , Y. Sun <sup>2</sup> , C. Wang <sup>3</sup> , C. Baldwin <sup>4</sup> , P. Nara <sup>5</sup> , J. Trujillo <sup>6</sup> ; <sup>1</sup> Department of Clinical Sciences, Mississippi State University College of Veterinary Medicine, Starkville, MS, USA, <sup>2</sup> Department of Statistics, Iowa State University, Ames, IA, USA, <sup>3</sup> Department of Veterinary Diagnostic and Production Animal Medicine, Iowa State University College of Veterinary Medicine, Ames, IA, USA, <sup>4</sup> Department of Veterinary Clinical Sciences, Iowa State University College of Veterinary Medicine, Ames, IA, USA, <sup>5</sup> Biological Mimetics, Inc., Frederick, MD, USA, <sup>6</sup> Department of Veterinary Microbiology and Preventative Medicine, Iowa State University College of Veterinary Medicine, Ames, IA, USA.
<b>8:45</b>	<b>034</b>	An insulated isothermal PCR-POCKIT <sup>®</sup> method for rapid and sensitive detection of canine parvovirus at the point of care	<b>R.P. Wilkes<sup>1</sup></b> , H.-H. Chang <sup>2</sup> , C.-F. Ping <sup>2</sup> , Y.-L. Tsai <sup>2</sup> , C.-F. Tsai <sup>2</sup> , P.-Y. Lee <sup>2</sup> , H.-F. Chang <sup>2</sup> , H.-T. Wang <sup>2</sup> ; <sup>1</sup> Biomedical and Diagnostic Sciences, The University of Tennessee College of Veterinary Medicine, Knoxville, TN, USA, <sup>2</sup> GeneReach USA, Lexington, MA, USA.
<b>9:00</b>	<b>035</b>	Molecular epidemiological analysis of MRSP environmental contamination in a veterinary hospital: Looking beyond infections and treatments	<b>J. van Balen</b> , A.E. Hoet; Veterinary Preventive Medicine, The Ohio State University, Columbus, OH, USA.
<b>9:15 Mon.</b>	<b>036</b>	Use of patient body temperatures in surveillance for healthcare-associated infections in a veterinary hospital.	<b>Z.B. Ouyang<sup>1</sup></b> , B.A. Burgess <sup>2</sup> , P.S. Morley <sup>1</sup> ; <sup>1</sup> Colorado State University, Fort Collins, CO, USA <sup>2</sup> Virginia Tech Blacksburg VA USA
<b>9:30</b>		<b>Break and Table Top Exhibits – Foyer</b>	
		<b>Presiders: Laura Hungerford and Enrique Doster</b>	
<b>10:00 Mon.</b>	<b>037</b>	Non-fatal injury occurrence in southern California Thoroughbred racehorses 2009-2010	<b>A.E. Hill<sup>1</sup></b> , J.A. Blea <sup>2</sup> , R.M. Arthur <sup>3</sup> , C.W. McIlwraith <sup>4</sup> ; <sup>1</sup> California Animal Hlth & Food Safety Lab, University of California-Davis, Davis, CA, USA, <sup>2</sup> Southern California Equine Foundation, Arcadia, CA, USA, <sup>3</sup> School of Veterinary Medicine, University of California-Davis, Davis, CA, USA, <sup>4</sup> Equine Orthopaedic Research Center, Dept of Clinical Sciences, Colorado State University, Fort Collins, CO, USA

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**COMPANION ANIMAL EPIDEMIOLOGY**  
**Michigan/Michigan State Room - 6th Floor**

**Section Leader: Audrey Ruple-Czerniak and Laura Hungerford**

<b>Time</b>	<b>No.</b>	<b>Title</b>	<b>AuthorBlock</b>
<b>10:15 Mon.</b>	<b>038</b>	Factors associated with long-term survival of geriatric horses in the U.S.	<b>N.T. Saklou<sup>1</sup></b> , B.A. Burgess <sup>2</sup> , P.S. Morley <sup>1</sup> , J.R. Gold <sup>3</sup> ; <sup>1</sup> Clinical Sciences, Colorado State University, Fort Collins, CO, USA, <sup>2</sup> Population Health Sciences, Virginia Tech, Blacksburg, VA, USA, <sup>3</sup> Chaparral Equine, Cave Creek, AZ, USA.
<b>10:30</b>	<b>039</b>	Canine B-cell chronic lymphocytic leukemia shows strong breed-specific risk	<b>J.L. Bromberek<sup>1</sup></b> , M.R. Agnew <sup>1</sup> , P.S. Morley <sup>2</sup> , A.C. Avery <sup>1</sup> ; <sup>1</sup> Microbiology, Immunology, and Pathology, Colorado State University, Fort Collins, CO, USA, <sup>2</sup> Clinical Sciences, Colorado State University, Fort Collins, CO, USA.
<b>10:45</b>	<b>040</b>	Multicenter case-control investigation of risks associated with development of canine lymphoma in North America: 18,826 cases (1990-2009)	<b>A. Ruple</b> , P. Morley; Colorado State University, Fort Collins, CO, USA.
<b>11:00 Mon.</b>	<b>041</b>	Differences in the geographic distribution of B-cell and T-zone lymphomas in Golden retrievers in the United States	<b>A. Ruple</b> , A. Avery, P. Morley; Colorado State University, Fort Collins, CO, USA.
<b>11:15</b>		Open	
<b>11:30</b>		<b>Lunch Break</b>	
<b>4:30 to</b>	<b>5:00</b>	<b>Break and Table Top Exhibits – Foyer</b>	
<b>5:00 to</b>	<b>6:30</b>	<b>Poster Session II Grand Ballroom Salon III - 7th floor</b>	

**ECOLOGY AND MANAGEMENT OF FOODBORNE AGENTS**

**Salon E - 5th Floor**

**Section Leader: Yvette Johnson-Walker**

<b>Time</b>	<b>No.</b>	<b>Title</b>	<b>Authors</b>
<b>11:30</b>		<b>Lunch Break</b>	
<b>1:30 Mon.</b>	<b>043</b>	Campylobacter jejuni isolated from cattle in Michigan: genetic diversity, antimicrobial resistance, and the impact on public health	<b>W. Cha<sup>1</sup></b> , R. Mosci <sup>2</sup> , S. Wengert <sup>2</sup> , C. Venegas <sup>3</sup> , P. Bartlett <sup>3</sup> , D. Grooms <sup>3</sup> , S.D. Manning <sup>2</sup> ; <sup>1</sup> Comparative Medicine and Integrative Biology, Michigan State University, East Lansing, MI, USA, <sup>2</sup> Microbiology and Molecular Genetics, Michigan State University, East Lansing, MI, USA, <sup>3</sup> Large Animal Clinical Sciences, Michigan State University, East Lansing, MI, USA.
<b>1:45</b>	<b>044</b>	An evaluation of the impact of litter chemical amendments on reducing Campylobacter jejuni in broilers	<b>I.I. Kassem</b> , O.O. Kehinde, A. Kumar, R. Pina-Mimbela, K. Chandrashekhar, G. Rajashekara; Food Animal Health Research Program, The Ohio State University, Wooster, OH, USA.

# **ECOLOGY AND MANAGEMENT OF FOODBORNE AGENTS**

**Salon E - 5th Floor**

**Section Leader: Yvette Johnson-Walker**

<b>Time</b>	<b>No.</b>	<b>Title</b>	<b>Authors</b>
<b>2:00 Mon.</b>	<b>045</b>	Summer and winter prevalence of O26, O45, O103, O111, O121, O145 and O157 Shiga toxin-producing <i>Escherichia coli</i> (STEC) in feces of feedlot cattle	<b>D.M.A. Dewsbury</b> , D.G. Renter, T.G. Nagaraja, P.B. Shridhar, L.W. Noll, X. Shi, N. Cernicchiaro; Diagnostic Medicine and Pathobiology, Kansas State University, Manhattan, KS, USA.
<b>2:15</b>	<b>046</b>	Feedlot- and pen-level prevalence of Shiga toxin-producing <i>Escherichia coli</i> in feces of commercial feedlot cattle	<b>C.A. Cull</b> , D.G. Renter, D.M. Dewsbury, L.W. Noll, P.B. Shridhar, X. Shi, T.G. Nagaraja, N. Cernicchiaro; Diagnostic Medicine/Pathobiology, Kansas State University, Manhattan, KS, USA.
<b>2:30</b>	<b>047</b>	Prevalence of targeted enterohemorrhagic <i>Escherichia coli</i> in culled dairy cows	<b>Z.R. Stromberg</b> <sup>1</sup> , G.L. Lewis <sup>1</sup> , S.S. Aly <sup>2</sup> , T.W. Lehenbauer <sup>2</sup> , J.M. Bosilevac <sup>3</sup> , N. Cernicchiaro <sup>4</sup> , R.A. Moxley <sup>1</sup> ; <sup>1</sup> University of Nebraska - Lincoln, Lincoln, NE, USA, <sup>2</sup> University of California - Davis, Tulare, CA, USA, <sup>3</sup> Roman L. Hruska U.S. Meat Animal Research Center, Clay Center, NE, USA, <sup>4</sup> University of Kansas State, Manhattan, KS, USA.
<b>2:45</b>		<b>Break and Table Top Exhibits – Foyer</b>	
<b>3:00 Mon.</b>	<b>048</b>	Quantification of six non-O157 <i>E. coli</i> serogroups in cattle feces by spiral plating method	<b>P. Belagola Shridhar</b> <sup>1</sup> , L. Noll <sup>1</sup> , E. Kim <sup>1</sup> , C. Cull <sup>1</sup> , D. Dewsbury <sup>1</sup> , X. Shi <sup>1</sup> , N. Cernicchiaro <sup>1</sup> , D.G. Renter <sup>1</sup> , J. Bai <sup>2</sup> , T.G. Nagaraja <sup>1</sup> ; <sup>1</sup> College Of Veterinary Medicine, Manhattan, KS, USA, <sup>2</sup> Pathobiology, Veterinary Diagnostic Laboratory, Manhattan, KS, USA.
<b>3:15</b>	<b>049</b>	Modeling the intestinal concentrations of antimicrobials in animals	<b>V. Volkova</b> <sup>1</sup> , C. Cazer <sup>2</sup> , Y.T. Grohn <sup>2</sup> ; <sup>1</sup> Department of Diagnostic Medicine/Pathobiology, College of Veterinary Medicine, Kansas State University, Manhattan, KS, USA, <sup>2</sup> Department of Population Medicine and Diagnostic Sciences, College of Veterinary Medicine, Cornell University, Ithaca, NY, USA.
<b>3:30 Mon.</b>	<b>050</b>	Effect of heifer-raising practices on <i>E. coli</i> antimicrobial resistance and <i>Salmonella</i> prevalence among heifer fecal pats.	<b>R.V. Pereira</b> <sup>1</sup> , J.D. Siler <sup>1</sup> , K.J. Cummings <sup>2</sup> , M.A. Davis <sup>3</sup> , L.D. Warnick <sup>1</sup> ; <sup>1</sup> College of Veterinary Medicine - Population Medicine and Diagnostic Sciences (VTPMD), Cornell University, Ithaca, NY, USA, <sup>2</sup> College of Vet. Medicine and Biomedical Sciences, Department of Veterinary Integrative Biosciences, Texas A&M University, College Station, TX, USA, <sup>3</sup> College of Veterinary Medicine, Washington State University, Pullman, WA, USA.

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# **ECOLOGY AND MANAGEMENT OF FOODBORNE AGENTS**

**Salon E - 5th Floor**

**Section Leader: Yvette Johnson-Walker**

<b>Time</b>	<b>No.</b>	<b>Title</b>	<b>Authors</b>
<b>3:45 Mon.</b>	<b>051</b>	Using metagenomics to unlock the ecology of antimicrobial resistance in cattle production systems	<b>N. Noyes</b> <sup>1</sup> , Y. Xiang <sup>2</sup> , J. McArt <sup>3</sup> , L.M. Linke <sup>1</sup> , R.J. Magnuson <sup>1</sup> , H. Yang <sup>2</sup> , A. Dettenwanger <sup>4</sup> , K.L. Jones <sup>5</sup> , C. Boucher <sup>4</sup> , K.E. Belk <sup>2</sup> , P.S. Morley <sup>1</sup> ; <sup>1</sup> Clinical Sciences, Colorado State University, Fort Collins, CO, USA, <sup>2</sup> Animal Sciences, Colorado State University, Fort Collins, CO, USA, <sup>3</sup> Cornell University, Ithaca, NY, USA, <sup>4</sup> Computer Sciences, Colorado State University, Fort Collins, CO, USA, <sup>5</sup> Biochemistry and Molecular Genetics, University of Colorado, Denver, CO, USA.
<b>4:00</b>	<b>052</b>	Enterobacteriaceae producing extended spectrum beta-lactamases from wild birds on Ohio dairies.	<b>D.A. Mathys</b> <sup>1</sup> , B.A. Mathys <sup>2</sup> , A.E. Strait <sup>1</sup> , D.F. Mollenkopf <sup>1</sup> , J.B. Daniels <sup>3</sup> , T.E. Wittum <sup>1</sup> ; <sup>1</sup> Department of Veterinary Preventative Medicine, College of Veterinary Medicine, Ohio State University, Columbus, OH, USA, <sup>2</sup> Department of Natural Sciences, Ohio Dominican University, Columbus, OH, USA, <sup>3</sup> Department of Veterinary Clinical Sciences, College of Veterinary Medicine, Ohio State University, Columbus, OH, USA
<b>4:15 Mon.</b>	<b>053</b>	Prevalence and characteristics of Salmonella found on the paws and in the feces of free-ranging raccoons (Procyon lotor) in southern Ontario, Canada.	<b>K.J. Bondo</b> <sup>1</sup> , D.L. Pearl <sup>2</sup> , N. Janecko <sup>3</sup> , P. Boerlin <sup>1</sup> , R.J. Reid-Smith <sup>3</sup> , J. Parmley <sup>3</sup> , C.M. Jardine <sup>4</sup> ; <sup>1</sup> Department of Pathobiology, University of Guelph, Guelph, ON, Canada, <sup>2</sup> Department of Population Medicine, University of Guelph, Guelph, ON, Canada, <sup>3</sup> Laboratory of Foodborne Zoonoses, Public Health Agency of Canada, Guelph, ON, Canada, <sup>4</sup> Canadian Wildlife Health Cooperative, Department of Pathobiology, University of Guelph, Guelph, ON, Canada
<b>4:30 to 5:00</b>	<b>5:00</b>	<b>Break and Table Top Exhibits – Foyer</b>	
<b>5:00 to</b>	<b>6:30</b>	<b>Poster Session II Grand Ballroom Salon III - 7th floor</b>	

**ECOLOGY AND MANAGEMENT OF FOODBORNE AGENTS**  
**Salon E - 5th Floor - Section Leader: Yvette Johnson-Walker**

Time	No.	Title	Authors
<b>8:00-8:45</b> <b>Tues.</b> Keynote	<b>084</b>	<b>Epidemiology and Animal Health Economics Keynote- Salons A/B/C/D:</b> The Symbiology of Epidemiologic Pursuits of Academia and Government.	<b>B.J. McCluskey</b> ; Chief Epidemiologist, USDA-APHIS-Veterinary Service, Fort Collins, CO, USA.
<b>8:45</b>	<b>085</b>	<b>Mark Gearhart Memorial Award, Salons A/B/C/D:</b> Mannheimia haemolytica in feedlot cattle: associations with antimicrobial use, resistance and health outcomes	<b>N.R. Noyes</b> <sup>1</sup> , K.M. Benedict <sup>1</sup> , S.P. Gow <sup>2</sup> , C.W. Booker <sup>3</sup> , S.J. Hannon <sup>3</sup> , T.A. McAllister <sup>4</sup> , P.S. Morley <sup>1</sup> ; <sup>1</sup> Clinical Sciences, Colorado State University, Fort Collins, CO, USA, <sup>2</sup> Laboratory for Foodborne Zoonoses, University of Saskatchewan, Saskatoon, SK, Canada, <sup>3</sup> Feedlot Health Management Services, Okotoks, AB, Canada, <sup>4</sup> Lethbridge Research Center, University of Lethbridge, Lethbridge, AB, Canada
<b>9:00</b>	<b>054</b>	Potential transfer of antimicrobial resistance (AMR) Salmonella and Staphylococcus sciuri after application of swine manure in the environment	<b>S. Pornsukarom</b> , S. Thakur; College of Veterinary Medicine, North Carolina State University, Raleigh, NC, USA.
<b>9:15</b> <b>Tues.</b>	<b>055</b>	Risk factors for antimicrobial resistance of Escherichia coli isolates from Ontario broiler chicken flocks at chick placement: A comparison of three production system types	T.E. Roberts <sup>1</sup> , S.A. McEwen <sup>1</sup> , R. Reid-Smith <sup>2</sup> , J.M. Sargeant <sup>1</sup> , A. Agunos <sup>2</sup> , D. Léger <sup>2</sup> , <b>M.T. Guerin</b> <sup>1</sup> ; <sup>1</sup> Population Medicine, University of Guelph, Guelph, ON, Canada, <sup>2</sup> Public Health Agency of Canada, Guelph, ON, Canada.
<b>9:30</b>		<b>Break – Foyer</b>	
<b>10:00</b>	<b>056</b>	Predictors for contamination of informally traded ready-to-eat (RTE) chicken with generic (Biotype I) Escherichia coli	<b>J.W. Oguttu</b> <sup>1</sup> , C.M.E. McCrindle <sup>2</sup> , F.O. Fasina <sup>3</sup> ; <sup>1</sup> School of life and Consumer Sciences, Department of Agriculture and Animal Health, University of South Africa, Pretoria, South Africa, <sup>2</sup> School of Health Systems and Public Health, Faculty of Health Sciences, University of South Pretoria, Pretoria, South Africa, <sup>3</sup> cUnit of Swine Health and Production, Department of Production Animals Studies, University of Pretoria, Pretoria, South Africa.
<b>10:15</b> <b>Tues.</b>	<b>057</b>	Salmonella phenotypic and genotypic diversity in finishing swine	<b>A. Pires</b> <sup>1</sup> , J. Funk <sup>2</sup> , G. Habing <sup>3</sup> , C. Bolin <sup>4</sup> ; <sup>1</sup> Medicine & Epidemiology, School of Veterinary Medicine, University of California, Davis, Davis, CA, USA, <sup>2</sup> Large Animal Clinical Sciences, College of Veterinary Medicine, Michigan State University, East Lansing, MI, USA, <sup>3</sup> Veterinary Preventive Medicine, College of Veterinary Medicine, The Ohio State University, Columbus, OH, USA, <sup>4</sup> Diagnostic Center for Population and Animal Health, College of Veterinary Medicine, Michigan State University, East Lansing, MI, USA.



# **ECOLOGY AND MANAGEMENT OF FOODBORNE AGENTS**

**Salon E - 5th Floor**

**Section Leader: Yvette Johnson-Walker**

<b>Time</b>	<b>No.</b>	<b>Title</b>	<b>Authors</b>
<b>10:30 Tues.</b>	<b>058</b>	Oral <i>Salmonella</i> challenge and subsequent uptake by the peripheral lymph nodes in calves	<b>T.R. Brown<sup>1</sup></b> , T.S. Edrington <sup>1</sup> , K.J. Genovese <sup>1</sup> , G.H. Loneragan <sup>2</sup> , D.J. Nisbet <sup>1</sup> ; <sup>1</sup> U.S. Department of Agriculture, Agricultural Research Service, Food and Feed Safety Research Unit, College Station, TX, USA, <sup>2</sup> International Center for Food Industry Excellence, Department of Animal and Food Sciences, Texas Tech University, Lubbock, TX, USA.
<b>10:45</b>	<b>059</b>	High throughput environmental testing for <i>Salmonella</i> sp. using Matrix-Assisted Laser Desorption/Ionization-Time of Flight MALDI-TOF) technology	<b>K.A. Clothier</b> , A. Torain, C. Hult, N. Saechao, K. Kumimoto, J. Baltezore, S. Reinl, M. Wright, A. Sutsakhan, M. Villanueva; California Animal Health & Food Safety Lab, University of California, Davis, Davis, CA, USA.
<b>11:00</b>	<b>060</b>	Designing a risk communication strategy for health hazards posed by traditional slaughter of goats in Tshwane, South Africa	D.N. Qekwana <sup>1</sup> , C.M.E. McCrindle <sup>2</sup> , <b>J.W. Oguttu<sup>3</sup></b> ; <sup>1</sup> Paraclinical Sciences, Section VPH, University of Pretoria, Pretoria, South Africa, <sup>2</sup> School of Health Systems and Public Health, University of Pretoria, Pretoria, South Africa, <sup>3</sup> Agriculture and Animal Health, University of South Africa, Pretoria, South Africa.
<b>11:15 Tues.</b>	<b>061</b>	Evidence from bioassays that commercial spray drying processes are effective at inactivating porcine epidemic diarrhea virus	<b>T. Opriessnig<sup>1</sup></b> , C.-T. Xiao <sup>2</sup> , P.F. Gerber <sup>1</sup> , Q. Chen <sup>2</sup> , J. Zhang <sup>2</sup> , P.G. Halbur <sup>2</sup> ; <sup>1</sup> The Roslin Institute, University of Edinburgh, Midlothian, UK <sup>2</sup> Iowa State University Ames, IA, USA
<b>11:45 to</b>	<b>12:30</b>	<b>Business Meeting, Dedication, New Members Introduction, and Graduate Student Competition Awards Presentations</b>	

# **EPIDEMIOLOGY AND ANIMAL HEALTH ECONOMICS**

**Salons A/B/C/D - 5th Floor**

**Section Leader: Ashley Hill**

Time	No.	Title	Author
8:00 Mon.	062	Estimating the Number of Human Cases of Ceftiofur-Resistant <i>Salmonella enterica</i> serovar Heidelberg in Québec and Ontario, Canada (2003-2011)	<b>S.J.G. Otto</b> <sup>1</sup> , C.A. Carson <sup>2</sup> , R.L. Finley <sup>3</sup> , M.K. Thomas <sup>3</sup> , R.J. Reid-Smith <sup>2</sup> , S.A. McEwen <sup>1</sup> ; <sup>1</sup> Department of Population Medicine, Ontario Veterinary College, University of Guelph, Guelph, ON, Canada, <sup>2</sup> Laboratory for Foodborne Zoonoses, Public Health Agency of Canada, Guelph, ON, Canada, <sup>3</sup> Centre for Food-borne, Environmental and Zoonotic Infectious Diseases, Public Health Agency of Canada, Guelph, ON, Canada.
8:15	063	Extended-spectrum cephalosporin resistant nontyphoidal <i>Salmonella</i> recovered from clinical human infections in Ohio, USA.	<b>D. Mollenkopf</b> <sup>1</sup> , C. King <sup>1</sup> , D. Mathys <sup>1</sup> , S. Kim <sup>1</sup> , R. Adams <sup>1</sup> , E. Brandt <sup>2</sup> , T. Wittum <sup>1</sup> ; <sup>1</sup> Veterinary Preventive Medicine, Ohio State University, Columbus, OH, USA, <sup>2</sup> Ohio Department of Health, Reynoldsburg, OH, USA.
8:30	064	Understanding the occurrence of <i>Escherichia coli</i> O157:H7 super-shedding infections in feedlot cattle	<b>E. Antaki</b> <sup>1</sup> , X. Li <sup>1</sup> , B. Hoar <sup>2</sup> , J. Adaska <sup>3</sup> , B. Byrne <sup>3</sup> , E. Atwill <sup>1</sup> ; <sup>1</sup> Population Health and Reproduction; Western Institute for Food Safety and Security, University of California, Davis, CA, USA, <sup>2</sup> College of Agriculture and Natural Resources, University of Wyoming, Laramie, WY, USA, <sup>3</sup> Pathology, Microbiology & Immunology, University of California, Davis, CA, USA.
8:45	065	Effect of feeding preweaned dairy calves raw milk with residual concentrations of antimicrobials on the resistance of commensal fecal <i>Escherichia coli</i> .	<b>R.V. Pereira</b> , J.D. Siler, S. Ruback, R.C. Bicalho, L.D. Warnick; College of Veterinary Medicine - Population Medicine and Diagnostic Sciences (VTPMD), Cornell University, Ithaca, NY, USA.
9:00	066	Longitudinal study of nasal carriage of <i>Staphylococcus aureus</i> in swine veterinarians and its implications for health	<b>J. Sun</b> , S. Sreevatsan, M. Yang, P.R. Davies; University of Minnesota, St. Paul, MN, USA.
9:15	067	Bile salt hydrolase: a microbiome target for enhanced animal health	<b>R. Negga</b> , X. Zeng, K. Smith, J. Lin; University of Tennessee, Knoxville, TN, USA.
9:30		<b>Break and Table Top Exhibits – Foyer</b>	
10:00 Mon.	068	Investigating the efficacy of antimicrobial metaphylaxis in finishing pigs	<b>C. Ramirez</b> <sup>1</sup> , A. Harding <sup>2</sup> , E. Forteguerra <sup>1</sup> , B. Aldridge <sup>1</sup> , J. Lowe <sup>1</sup> ; <sup>1</sup> Veterinary Medicine, University of Illinois, Urbana, IL, USA, <sup>2</sup> Lowe Consulting, Ltd. Albers, IL, USA.

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# **EPIDEMIOLOGY AND ANIMAL HEALTH ECONOMICS**

**Salons A/B/C/D - 5th Floor**

**Section Leader: Ashley Hill**

<b>Time</b>	<b>No.</b>	<b>Title</b>	<b>Author</b>
<b>10:15 Mon.</b>	<b>069</b>	Role of direct and indirect transmission of different PRRSV genotypes within and between swine production systems in the US	A.F.A. Pires <sup>1</sup> , D. Polson <sup>2</sup> , R. Main <sup>3</sup> , E. Mondaca-Fernandez <sup>2</sup> , E. Johnson <sup>2</sup> , D. Holtkamp <sup>3</sup> , K. Mueller <sup>3</sup> , Z. Whedbee <sup>1</sup> , A. Perez <sup>4</sup> , <b>B. Martinez Lopez</b> <sup>1</sup> ; <sup>1</sup> Medicine and Epidemiology, University of California, Davis, Davis, CA, USA, <sup>2</sup> Boehringer-Ingelheim Vetmedica, Inc, Ames, IA, USA, <sup>3</sup> Veterinary Diagnostic and Production Animal Medicine, Iowa State University, Ames, IA, USA, <sup>4</sup> Veterinary Population Medicine, University of Minnesota, St Paul, MN, USA.
<b>10:30</b>	<b>070</b>	Disease investigation using data from a PRRS area regional control and elimination (ARC&E) project in Ontario, Canada.	<b>A.G. Arruda</b> <sup>1</sup> , R. Friendship <sup>1</sup> , J. Carpenter <sup>2</sup> , K. Hand <sup>3</sup> , D. Ojkic <sup>4</sup> , Z. Poljak <sup>1</sup> ; <sup>1</sup> Population Medicine, University of Guelph, Guelph, ON, Canada, <sup>2</sup> Ontario Swine Health Advisory Board, Stratford, ON, Canada, <sup>3</sup> Strategic Solutions Group, Puslinch, ON, Canada, <sup>4</sup> Animal Health Laboratory, University of Guelph, Guelph, ON, Canada.
<b>10:45</b>	<b>071</b>	Behavioral aspects of oral fluid sample collection	<b>A. Holmes</b> <sup>1</sup> , A. Kittawornrat <sup>1</sup> , C. Goodell <sup>2</sup> , Y. Panyasing <sup>1</sup> , S. Hoff <sup>3</sup> , K. Subramanya <sup>3</sup> , J. Zimmerman <sup>1</sup> , C. Wang <sup>1</sup> ; <sup>1</sup> Veterinary Diagnostics and Production Animal Medicine, Iowa State University, Ames, IA, USA, <sup>2</sup> IDEXX Laboratories, Westbrook, ME, USA, <sup>3</sup> Agriculture and Biosystems Engineering, Iowa State University, Ames, IA, USA.
<b>11:00</b>	<b>072</b>	Influenza H1N1 and H3N2 co-infection in pigs afterweaning.	<b>C.A. Diaz</b> , M. Culhane, S. Sreevatsan, M. Torremorell; Dept of Veterinary Population Medicine, College of Veterinary Medicine, University of Minnesota, Saint Paul, MN, USA.
<b>11:15</b>		Open	
<b>11:30</b>		<b>Lunch Break</b>	
<b>Time</b>	<b>No.</b>	<b>Title</b>	<b>Author</b>
<b>1:30 Mon.</b>	<b>073</b>	Smartphone applications for veterinary data collection in Malaysia: feasibility and data usage in animal disease surveillance	<b>N. Amirah Ahmad Ghani</b> <sup>1</sup> , B. Martinez Lopez <sup>2</sup> , A.F.A. Pires <sup>2</sup> , S.A. Rahman <sup>3</sup> , M.S.S. Omar <sup>1</sup> ; <sup>1</sup> Universiti Teknologi Malaysia, Johor, Malaysia, <sup>2</sup> Medicine and Epidemiology, University of California, Davis, Davis, CA, USA, <sup>3</sup> Johor Southern Regional Veterinary Laboratory, Johor, Malaysia.

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# **EPIDEMIOLOGY AND ANIMAL HEALTH ECONOMICS**

**Salons A/B/C/D - 5th Floor**

**Section Leader: Ashley Hill**

<b>Time</b>	<b>No.</b>	<b>Title</b>	<b>Author</b>
<b>1:45 Mon.</b>	<b>074</b>	Characterization of the network of live fish movements in the Irish salmon farming industry and implications for disease prevention and control	<b>T. Yatabe</b> <sup>1</sup> , S.J. More <sup>2</sup> , F. Geoghegan <sup>3</sup> , C. McManus <sup>4</sup> , A. Hill <sup>1</sup> , B. Martinez-Lopez <sup>1</sup> ; <sup>1</sup> Medicine and Epidemiology, University of California Davis, Davis, CA, USA, <sup>2</sup> University College Dublin, Dublin, Ireland, <sup>3</sup> Marine Institute, Galway, Ireland, <sup>4</sup> Marine Harvest Ireland, Binnmore, Ireland
<b>2:00</b>	<b>075</b>	A qualitative risk model for animal welfare concerns due to movement restrictions during a classical swine fever (CSF) outbreak	<b>S. Yadav</b> <sup>1</sup> , M. Ash <sup>2</sup> , M. Cooper <sup>2</sup> , C. Croney <sup>1</sup> , N. Olynk Widmar <sup>3</sup> , H.Y. Weng <sup>1</sup> ; <sup>1</sup> Comparative Pathobiology, Purdue University, West Lafayette, IN, USA, <sup>2</sup> Indiana State Board of Animal Health, Indianapolis, IN, USA, <sup>3</sup> Agricultural Economics, Purdue University, West Lafayette, IN, USA
<b>2:15</b>	<b>076</b>	The Alberta Veterinary Surveillance Network: Creating a tool to track cattle diseases and movements in Alberta, Canada	<b>H. Izakian</b> <sup>1</sup> , J. Li <sup>1</sup> , S. Otto <sup>2</sup> , C. Pollock <sup>2</sup> , D. Peters <sup>2</sup> , J. Patel <sup>2</sup> , C. Morley <sup>2</sup> , I. Jamal <sup>3</sup> , M. Reformat <sup>1</sup> , J. Berezowski <sup>4</sup> , W. Pedrycz <sup>1</sup> ; <sup>1</sup> Department of Electrical and Computer Engineering, University of Alberta, EDMONTON, AB, Canada, <sup>2</sup> Animal Health and Assurance Division, Alberta Agriculture and Rural Development, EDMONTON, AB, Canada, <sup>3</sup> AQL Management Consulting Ltd., EDMONTON, AB, Canada, <sup>4</sup> Veterinary Public Health Institute, Vetsuisse Fakultät, University of Guelph, Guelph, ON, Canada.
<b>2:30</b>	<b>077</b>	Using scan statistics to explore the relative performance of dead birds and mosquito pools in surveillance for West Nile virus in Ontario, 2002-2008.	<b>A.L. Thomas-Bachli</b> <sup>1</sup> , D.L. Pearl <sup>1</sup> , O. Berke <sup>1</sup> , J. Parmley <sup>2</sup> , I.K. Barker <sup>3</sup> ; <sup>1</sup> Department of Population Medicine, University of Guelph, Guelph, ON, Canada, <sup>2</sup> Canadian Wildlife Health Cooperative, Guelph, ON, Canada, <sup>3</sup> Department of Pathobiology, University of Guelph, Guelph, ON, Canada.
<b>2:45</b>		<b>Break and Table Top Exhibits – Foyer</b>	
<b>3:00 Mon.</b>	<b>078</b>	A meta-analysis of the effects of feeding active dry yeast of <i>Saccharomyces cerevisiae</i> , on milk production of lactating dairy cows	<b>G.D. Poppy</b> , A. Ruple-Czerniak, P.S. Morley; Clinical Science, Colorado State University, Fort Collins, CO, USA.
<b>3:15</b>	<b>079</b>	Dairy cattle management factors that influence on-farm density of European starlings ( <i>Sturnus vulgaris</i> ) in Ohio, 2007 - 2009	<b>G.A. Medhanie</b> <sup>1</sup> , D.L. Pearl <sup>1</sup> , S.A. McEwen <sup>1</sup> , M.T. Guerin <sup>1</sup> , C.M. Jardine <sup>2</sup> , J.T. LeJeune <sup>3</sup> ; <sup>1</sup> Population Medicine, University of Guelph, Guelph, ON, Canada, <sup>2</sup> Pathobiology, University of Guelph, Guelph, ON, Canada, <sup>3</sup> Food Animal and Health Research Program, The Ohio State University, Wooster, OH, USA.

# **EPIDEMIOLOGY AND ANIMAL HEALTH ECONOMICS**

**Salons A/B/C/D - 5th Floor**

**Section Leader: Ashley Hill**

<b>Time</b>	<b>No.</b>	<b>Title</b>	<b>Author</b>
<b>3:30 Mon.</b>	<b>080</b>	Spatial clustering of bovine tuberculosis outbreaks in Uruguay.	<b>C. Picasso</b> <sup>1</sup> , A. Perez <sup>1</sup> , S. Wells <sup>1</sup> , F. Fernandez <sup>2</sup> , A. Gil <sup>3</sup> ; <sup>1</sup> Veterinary Population Medicine, University of Minnesota, St Paul, MN, USA, <sup>2</sup> Animal Health Bureau, Ministry of Livestock, Agriculture and Fisheries, Montevideo, Uruguay, <sup>3</sup> Facultad de Veterinaria, Universidad de la Republica, Montevideo, Uruguay
<b>3:45</b>	<b>081</b>	The effect of morbidity on weaning weight of beef calves	<b>L.G. Schneider</b> , D.R. Smith; College of Veterinary Medicine, Mississippi State University, Mississippi State, MS, USA.
<b>4:00</b>	<b>082</b>	Performance of FAMACHA scores for detecting anemia in sheep	<b>K. Barton</b> <sup>1</sup> , J. Ondrak <sup>2</sup> , K. Shuck <sup>2</sup> , D. Smith <sup>1</sup> ; <sup>1</sup> College of Veterinary Medicine, Mississippi State University, Mississippi State, MS, USA, <sup>2</sup> Great Plains Veterinary Educational Center, University of Nebraska-Lincoln, Clay Center, NE, USA
<b>4:15 Mon.</b>	<b>083</b>	Respiratory disease outbreak detection within a population of free-living chimpanzees ( <i>Pan troglodytes schweinfurthii</i> )	<b>T. Wolf</b> <sup>1</sup> , D. Travis <sup>1</sup> , E. Lonsdorf <sup>2</sup> , I. Lipende <sup>3</sup> , T. Gillespie <sup>4</sup> , K. Terio <sup>5</sup> , B. Hahn <sup>6</sup> , A. Pusey <sup>7</sup> , C. Murray <sup>8</sup> , R. Singer <sup>1</sup> ; <sup>1</sup> University of Minnesota, St. Paul, MN, USA, <sup>2</sup> Franklin and Marshall College, Lancaster, PA, USA, <sup>3</sup> Greater Gombe Ecosystem Health Project, Gombe Stream National Park, Tanzania, United Republic of, <sup>4</sup> Emory University, Atlanta, GA, USA, <sup>5</sup> University of Illinois Zoological Pathology Program, Maywood, IL, USA, <sup>6</sup> University of Pennsylvania School of Medicine, Philadelphia, PA, USA, <sup>7</sup> Duke University, Durham, NC, USA, <sup>8</sup> Washington University, Washington, DC, USA.
<b>4:30 to 5:00</b>	<b>5:00</b>	<b>Break and Table Top Exhibits – Foyer</b>	
<b>5:00 to</b>	<b>6:30</b>	<b>Poster Session II Grand Ballroom Salon III - 7th floor</b>	

# **EPIDEMIOLOGY AND ANIMAL HEALTH ECONOMICS**

**Salons A/B/C/D - 5th Floor**

**Section Leader: Ashley Hill**

<b>Time</b>	<b>No.</b>	<b>Title</b>	<b>Authors</b>
<b>8:00-8:45 Tues. Keynote</b>	<b>084</b>	<b>Epidemiology and Animal Health Economics Keynote:</b> The Symbiology of Epidemiologic Pursuits of Academia and Government.	<b>B.J. McCluskey</b> ; Chief Epidemiologist, USDA-APHIS-Veterinary Service, Fort Collins, CO, USA.
<b>8:45</b>	<b>085</b>	<b>Mark Gearhart Memorial Award:</b> Mannheimia haemolytica in feedlot cattle: associations with antimicrobial use, resistance and health outcomes	<b>N.R. Noyes</b> <sup>1</sup> , K.M. Benedict <sup>1</sup> , S.P. Gow <sup>2</sup> , C.W. Booker <sup>3</sup> , S.J. Hannon <sup>3</sup> , T.A. McAllister <sup>4</sup> , P.S. Morley <sup>1</sup> ; <sup>1</sup> Clinical Sciences, Colorado State University, Fort Collins, CO, USA, <sup>2</sup> Laboratory for Foodborne Zoonoses, University of Saskatchewan, Saskatoon, SK, Canada, <sup>3</sup> Feedlot Health Management Services, Okotoks, AB, Canada, <sup>4</sup> Lethbridge Research Center, University of Lethbridge, Lethbridge, AB, Canada
<b>9:00</b>	<b>086</b>	Reporting guidelines for observational studies in veterinary medicine: STROBE-Vet	<b>J.M. Sargeant</b> <sup>1</sup> , I.R. Dohho <sup>2</sup> , H.N. Erb <sup>3</sup> , A.M. O'Connor <sup>4</sup> ; <sup>1</sup> Ontario Veterinary College, Guelph, ON, Canada, <sup>2</sup> Atlantic Veterinary College, Charlottetown, PE, Canada, <sup>3</sup> Cornell University College of Veterinary Medicine, Ithaca, NY, USA, <sup>4</sup> Iowa State University, Ames, IA, USA
<b>9:15</b>	<b>087</b>	Mathematical disease transmission models for livestock populations: A scoping review	<b>B. Goh, A. Greer</b> ; Population Medicine, Ontario Veterinary College, University of Guelph, Guelph, ON, Canada.
<b>9:30</b>		<b>Break</b>	
<b>10:00 Tues.</b>	<b>088</b>	Does exercise-induced pulmonary hemorrhage affect career longevity and performance among South African Thoroughbred racehorses?	<b>J.L. Bromberek</b> <sup>1</sup> , A.J. Guthrie <sup>2</sup> , K.W. Hinchcliff <sup>3</sup> , P.S. Morley <sup>4</sup> ; <sup>1</sup> Microbiology, Immunology, and Pathology, Colorado State University, Fort Collins, CO, USA, <sup>2</sup> Equine Research Centre, Faculty of Veterinary Science, University of Pretoria, Pretoria, South Africa, <sup>3</sup> Faculty of Veterinary Science, University of Melbourne, Victoria, Australia, <sup>4</sup> Clinical Sciences, Colorado State University, Fort Collins, CO, USA
<b>10:15</b>	<b>089</b>	Human Q fever: seroprevalence and exploration of risk factors for Coxiella burnetii exposure in small ruminant farm workers and veterinarians/veterinary students	<b>S. Meadows</b> <sup>1</sup> , <b>A. Jones-Bitton</b> <sup>1</sup> , J.T. Jansen <sup>2</sup> , S.A. McEwen <sup>1</sup> , S. Patel <sup>3</sup> , C. Filejski <sup>4</sup> , P.I. Menzies <sup>1</sup> ; <sup>1</sup> Department of Population Medicine, University of Guelph, Guelph, ON, Canada, <sup>2</sup> Veterinary Science and Policy, Ontario Ministry of Agriculture, Food and Rural Affairs, Elora, ON, Canada, <sup>3</sup> Public Health Ontario, Toronto, ON, Canada, <sup>4</sup> Ontario Ministry of Health and Long-Term Care, Toronto, ON, Canada.

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# **EPIDEMIOLOGY AND ANIMAL HEALTH ECONOMICS**

**Salons A/B/C/D - 5th Floor**

**Section Leader: Ashley Hill**

<b>Time</b>	<b>No.</b>	<b>Title</b>	<b>Authors</b>
<b>10:30 Tues.</b>	<b>090</b>	Rare disease epidemiology: results from the national scrapie prevalence study	<b>O. Berke</b> <sup>1</sup> , S. Leung <sup>1</sup> , J. Tang <sup>1</sup> , H. Ortegon <sup>2</sup> , H. Brown <sup>3</sup> , P. Menzies <sup>1</sup> ; <sup>1</sup> Population Medicine, University of Guelph, Guelph, ON, Canada, <sup>2</sup> Alberta Agriculture and Rural Development, Edmonton, AB, Canada, <sup>3</sup> Canadian Food Inspection Agency, Ottawa, ON, Canada.
<b>10:45</b>	<b>091</b>	Epidemiology of <i>Salmonella</i> spp. among feral pigs in Texas	<b>L.D. Rodriguez-Rivera</b> <sup>1</sup> , K.J. Cummings <sup>1</sup> , S.C. Rankin <sup>2</sup> , M.K. FitzSimon <sup>1</sup> , B.T. Mesenbrink <sup>3</sup> , B.R. Leland <sup>3</sup> , M.J. Bodenchuk <sup>3</sup> ; <sup>1</sup> Department of Veterinary Integrative Biosciences, College of Veterinary Medicine, Texas A&M University, College Station, TX, USA, <sup>2</sup> Department of Pathobiology, School of Veterinary Medicine, University of Pennsylvania, Philadelphia, PA, USA, <sup>3</sup> USDA-APHIS-Wildlife Services, San Antonio, TX, USA.
<b>11:00</b>	<b>092</b>	Risk mapping of Avian influenza in California using Multiple-criteria decision analysis.	A. Lainez Nuez <sup>1</sup> , A.F.A. Pires <sup>1</sup> , M. Pitesky <sup>2</sup> , B. Crossley <sup>3</sup> , A. Hill <sup>3</sup> , R. Gallardo <sup>2</sup> , W. Boyce <sup>4</sup> , <b>B. Martinez Lopez</b> <sup>1</sup> ; <sup>1</sup> Medicine and Epidemiology, University of California, Davis, Davis, CA, USA, <sup>2</sup> Population Health and Reproduction, University of California, Davis, Davis, CA, USA, <sup>3</sup> California Animal Health & Food Safety Laboratory System, University of California, Davis, Davis, CA, USA, <sup>4</sup> Pathology, Microbiology & Immunology, University of California, Davis, Davis, CA, USA.
<b>11:15 Tues.</b>	<b>093</b>	Within-farm spread of highly pathogenic avian influenza in Korean outbreaks of H5N1 and H5N8 virus types	<b>H. Yoon</b> , O.-K. Moon, J. Choi, W. Jeong, Y.-M. Kang, Y.-J. Lee, H.-M. Kang, Y.-S. Kim; Veterinary Epidemiology Division, Animal and Plant Quarantine Agency, Anyang, Korea, Republic of.
<b>11:45 to</b>	<b>12:30</b>	<b>Business Meeting, Dedication, New Members Introduction, and Graduate Student Competition Awards Presentations</b>	

**IMMUNOLOGY**  
**Salons F/G/H - 5th Floor**

**Section Leader: Laura C. Miller and Renukaradhya J. Gourapura**

Time	No.	Title	AuthorBlock
		<b>Presider: Aradhya Gourapura</b>	
<b>8:00 Mon.</b>	<b>095</b>	Evaluation of porcine epidemic diarrhea virus transmission and the immune response in growing pigs	<b>K. Crawford</b> <sup>1</sup> , K. Lager <sup>1</sup> , L. Miller <sup>1</sup> , C. Loving <sup>1</sup> , T. Opriessnig <sup>2</sup> , P. Gerber <sup>3</sup> ; <sup>1</sup> United States Department of Agriculture - Agricultural Research Service, National Animal Disease Center, Ames, IA, USA, <sup>2</sup> Veterinary Diagnostic and Production Animal Medicine, Iowa State University, Ames, IA, USA, <sup>3</sup> The Roslin Institute, University of Edinburgh, Midlothian, UK
<b>8:15</b>	<b>096</b>	In vitro evaluation of serological cross-reactivity and cross-neutralization between the U.S. PEDV original and variant strains	<b>Q. Chen</b> <sup>1</sup> , J.T. Thomas <sup>1</sup> , L.G. Giménez-Lirola <sup>1</sup> , P.C. Gauger <sup>1</sup> , J.M. Hardham <sup>2</sup> , V.J. Rapp-Gabrielson <sup>2</sup> , D. Madson <sup>1</sup> , D.R. Magstadt <sup>1</sup> , M.W. Welch <sup>1</sup> , H. Salzbrenner <sup>1</sup> , J. Zhang <sup>1</sup> ; <sup>1</sup> VDPAM, Iowa State University, Ames, IA, USA, <sup>2</sup> Zoetis, Kalamazoo, MI, USA.
<b>8:30</b>	<b>097</b>	Relationship between maternal immune status and neonatal protection against PEDV infection	<b>K. Poonsuk</b> <sup>1</sup> , L. Gimenez-Lirola <sup>1</sup> , W. Gonzalez <sup>1</sup> , J. Zhang <sup>1</sup> , Q. Chen <sup>1</sup> , L. Carrion <sup>2</sup> , C. Olsen <sup>3</sup> , R. Magtoto <sup>4</sup> , J. Johnson <sup>1</sup> , C. Wang <sup>1</sup> , D. Madson <sup>1</sup> , R. Main <sup>1</sup> , J. Zimmerman <sup>1</sup> , K.-J. Yoon <sup>1</sup> ; <sup>1</sup> Veterinary Diagnostic and Production Animal Medicine, Iowa State University, Ames, IA, USA, <sup>2</sup> School of Veterinary Medicine and Animal Husbandry, University of Sao Paulo, Sao Paulo, Brazil, <sup>3</sup> Veterinary Medicine, Iowa State University, Ames, IA, USA, <sup>4</sup> Veterinary Diagnostic Laboratory, Iowa State University, Ames, IA, USA.
<b>8:45</b>	<b>098</b>	Interaction of interferons and mTOR signaling underlying PRRSV infection	<b>Q. Liu</b> , R.R.R. Rowland, F. Blecha, Y. Sang; Anatomy and Physiology, Kansas State University, Manhattan, KS, USA.
<b>9:00 Mon.</b>	<b>099</b>	Predicting vaccine efficacy for food animals using the Epitope Content Comparison (EpiCC) tool: Application to PRRSV	<b>A.H. Gutierrez</b> <sup>1</sup> , C. Loving <sup>2</sup> , L. Moise <sup>3</sup> , W. Martin <sup>4</sup> , A.S. De Groot <sup>3</sup> ; <sup>1</sup> Institute for Immunology and Informatics, University of Rhode Island, Providence, RI, USA, <sup>2</sup> Virus and Prion Diseases Research Unit, NADC, USDA ARS, Ames, IA, USA, <sup>3</sup> Institute for Immunology and Informatics, University of Rhode Island & EpiVax, Inc., Providence, RI, USA, <sup>4</sup> EpiVax, Inc., Providence, RI, USA.

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**IMMUNOLOGY**  
**Salons F/G/H - 5th Floor**

**Section Leader: Laura C. Miller and Renukaradhya J. Gourapura**

<b>Time</b>	<b>No.</b>	<b>Title</b>	<b>AuthorBlock</b>
<b>9:15 Mon.</b>	<b>100</b>	Evaluation of cross-protection in Fosterera□ PRRSV vaccinated conventional swine challenged with a contemporary, heterologous lineage 9 PRRSV field isolate.	<b>D. Magstadt<sup>1</sup></b> , P. Gauger <sup>1</sup> , D. Madson <sup>1</sup> , E. Burrough <sup>1</sup> , P. Arruda <sup>1</sup> , K. Harmon <sup>1</sup> , A. Pillatzki <sup>2</sup> , Q. Chen <sup>1</sup> , J. Thomas <sup>1</sup> , J. Zhang <sup>1</sup> ; <sup>1</sup> Veterinary Diagnostic and Production Animal Medicine, Iowa State University, Ames, IA, USA, <sup>2</sup> Veterinary and Biomedical Sciences, South Dakota State University, Brookings, SD, USA.
<b>9:30</b>		<b>Break and Table Top Exhibits – Foyer</b>	
		<b>Presider: Kumaran Subbiah</b>	
<b>10:00</b>		Open	
<b>10:15</b>	<b>102</b>	Mucosal correlates of cross-protection for live-attenuated influenza virus vaccines in pigs.	H. Hughes <sup>1</sup> , A. Vincent <sup>1</sup> , S. Brockmeier <sup>1</sup> , D. Perez <sup>2</sup> , <b>C.L. Loving<sup>1</sup></b> ; <sup>1</sup> Respiratory Diseases of Swine, USDA-ARS-National Animal Disease Center, Ames, IA, USA, <sup>2</sup> University of Maryland College Park, MD, USA
<b>10:30 Mon.</b>	<b>103</b>	PLGA-Nanoparticle entrapped swine influenza virus peptides vaccine induces epitope specific cell-mediated immune response in pigs	<b>J. Hiremath</b> , K.-I. Kang, M. Elaish, B. Binjawadagi, K. Ouyang, S. Dhakal, C.-W. Lee, R. Gourapura; Food Animal Health Research Program, OARDC, Veterinary Preventive Medicine, The Ohio State University, Wooster, OH, USA.
<b>10:45</b>	<b>104</b>	Recovery and Stability of DNA and Protein Antigens Formulated with VaxLiant Adjuvants Utilizing in vitro and in vivo Testing	<b>L. Trygstad<sup>1</sup></b> , M. Inman <sup>1</sup> , M. Pfannenstiel <sup>1</sup> , W. Swafford <sup>2</sup> ; <sup>1</sup> Benchmark Biolabs, Inc., Lincoln, NE, USA, <sup>2</sup> AgriLabs, Inc, St. Joseph, MO, USA.
<b>11:00</b>	<b>105</b>	Bovine ocular and systemic immune responses to an intranasal recombinant <i>Moraxella bovis</i> cytotoxin subunit vaccine adjuvanted with polyacrylic acid	<b>J. Angelos<sup>1</sup></b> , M. Chigerwe <sup>1</sup> , J. Edman <sup>1</sup> , J. Hess <sup>2</sup> ; <sup>1</sup> Medicine and Epidemiology, University of California, Davis, Davis, CA, USA, <sup>2</sup> Cell Biology & Human Anatomy, University of California, Davis, Davis, CA, USA.
<b>11:15 Mon.</b>	<b>106</b>	Effect of VaxLiant adjuvants on efficacy of mucosal administration of plant-derived Newcastle Disease vaccines in chickens	<b>D.T. Petrik<sup>1</sup></b> , S.R. Webb <sup>2</sup> , W.S. Swafford <sup>3</sup> , T.J. Miller <sup>1</sup> ; <sup>1</sup> Benchmark Biolabs, Inc., Lincoln, NE, USA, <sup>2</sup> Dow AgroSciences, Indianapolis, IN, USA, <sup>3</sup> AgriLabs, St. Joseph, MO, USA.
<b>11:30</b>		<b>Lunch Break</b>	
		<b>Presider: Susan Eicher</b>	
<b>1:30-2:15 Mon. Keynote</b>	<b>107</b>	<b>Distinguished Veterinary Immunologist Keynote:</b> Challenges with Poultry Immunology and Disease Research: Immune reagent development and maintaining genetic breeds of poultry (continued)	<b>H. Lillehoj</b> ; Animal Biosciences and Biotechnology Laboratory, USDA, West Friendship, MD, USA.

**IMMUNOLOGY**  
**Salons F/G/H - 5th Floor**

**Section Leader: Laura C. Miller and Renukaradhya J. Gourapura**

<b>Time</b>	<b>No.</b>	<b>Title</b>	<b>AuthorBlock</b>
<b>2:15 Mon.</b>	<b>108</b>	Propagation of feline respiratory epithelial cells at the air-liquid interface - an in vitro model to study felid herpesvirus 1 in cats	<b>R.K. Nelli</b> , R.K. Maes, M. Kiupel, G.S. Hussey; Pathobiology and Diagnostic Investigation, Michigan State University, East Lansing, MI, USA.
<b>2:30</b>	<b>109</b>	The role of interferon in clearing primary Bovine Herpesvirus-1 (BHV-1) infections	<b>R. Osman</b> <sup>1</sup> , P. Gonzalez-Cano <sup>2</sup> , P. Griebel <sup>2</sup> ; <sup>1</sup> School of Public Health, University of Saskatchewan, Saskatoon, SK, Canada, <sup>2</sup> Vaccine and Infectious Disease Organization, University of Saskatchewan, Saskatoon, SK, Canada
<b>2:45</b>		<b>Break and Table Top Exhibits – Foyer</b>	
		<b>Presider: Crystal Loving</b>	
<b>3:00 Mon.</b>	<b>110</b>	Late-gestational nutrient restriction affects immune responsiveness of offspring in beef cattle.	<b>M.C. Heller</b> <sup>1</sup> , B. VanderLey <sup>2</sup> , K.N. Niederecker <sup>3</sup> , A.M. Meyer <sup>3</sup> ; <sup>1</sup> Veterinary Medicine and Epidemiology, University of California Davis, Davis, CA, USA, <sup>2</sup> Veterinary Medicine and Surgery, University of Missouri, Columbia, MO, USA, <sup>3</sup> Animal Science, University of Missouri, Columbia, MO, USA
<b>3:15</b>	<b>111</b>	Immune suppression in BLV-infected dairy cattle	<b>M.C. Frie</b> , P.C. Bartlett, P.M. Coussens; Department of Animal Science, Michigan State University, East Lansing, MI, USA.
<b>3:30</b>	<b>112</b>	Effects of conditioned media from Histophilus somni infected bovine brain endothelial cells on fibrin deposition and Factor Xa activity of bovine neutrophils	<b>J.J. Rivera Rivas</b> , C.J. Czuprynski; Pathobiological Sciences, UW-Madison, Madison, WI, USA.
<b>3:45</b>	<b>113</b>	Expression of inflammation-associated genes in circulating leukocytes and activity of indoleamine-2,3-dioxygenase in dairy cattle with acute puerperal metritis and bacteremia.	<b>B. Credille</b> <sup>1</sup> , A. Woolums <sup>2</sup> , T. Robertson <sup>3</sup> , D. Hurley <sup>1</sup> , M. Overton <sup>1</sup> , S. Giguere <sup>2</sup> ; <sup>1</sup> Population Health, University of Georgia, Athens, GA, USA, <sup>2</sup> Large Animal Medicine, University of Georgia, Athens, GA, USA, <sup>3</sup> Physiology and Pharmacology, University of Georgia, Athens, GA, USA
<b>4:00</b>	<b>114</b>	Oxylipid profiles in biological samples of dairy cows with coliform mastitis.	<b>V. Mavangira</b> , J.C. Gandy, L.M. Sordillo; Large Animal Clinical Sciences, Michigan State University, East Lansing, MI, USA.
<b>4:15 Mon.</b>	<b>115</b>	Bovine neutrophils produce neutrophil extracellular traps (NETs) in response to Salmonella serotype Typhimurium	<b>R. Matulle</b> , J. Figueiredo, N.A. Aulik, C. Czuprynski; Department of Pathobiological Sciences, School of Veterinary Medicine, University of Wisconsin-Madison, Madison, WI, USA.
<b>4:30 to 5:00</b>	<b>5:00</b>	<b>Break and Table Top Exhibits – Foyer</b>	
<b>5:00 to 6:30</b>		<b>Poster Session II Grand Ballroom Salon III - 7th floor</b>	

**IMMUNOLOGY**  
**Salons F/G/H - 5th Floor**

**Section Leader: Laura C. Miller and Renukaradhya J. Gourapura**

<b>Time</b>	<b>No.</b>	<b>Title</b>	<b>Authors</b>
		<b>AAVI Mini-Symposium: The Analysis and the Role of the Microbiome in Immunology</b>	
		<b>Presider: Laura Miller and Aradhya Gourapura</b>	
<b>8:00-8:30 Tues.</b>	<b>116</b>	The Microbiota-Gut-Brain Axis: Increasing recognition of its role in health and disease	<b>M. Lyte</b> ; Immunotherapeutics and Biotechnology, Texas Tech University Health Sciences Center, Abilene, TX, USA.
<b>8:30</b>	<b>117</b>	Recovery of the gut microbiota is disturbance-dependent	<b>H.K. Allen</b> , T. Looft, S.M.D. Bearson, T.B. Stanton; Food Safety and Enteric Pathogens Research Unit, National Animal Disease Center, Ames, IA, USA.
<b>8:45</b>	<b>118</b>	Developing tools to investigate the swine-associated butyrate-producing microbiota and its relationship to Salmonella shedding phenotype	<b>J. Trachsel</b> <sup>1</sup> , T. Looft <sup>2</sup> , S.M.D. Bearson <sup>2</sup> , T.B. Stanton <sup>2</sup> , H.K. Allen <sup>2</sup> ; <sup>1</sup> Interdepartmental Microbiology Doctoral Training Program, Iowa State University, Ames, IA, USA, <sup>2</sup> FSEP, ARS, Ames, IA, USA
<b>9:00-9:30</b>	<b>119</b>	Tailoring probiotics as immunomodulators to enhance neonatal mucosal immunity to rotavirus (RV) vaccines or alleviate RV diarrhea: Evaluation in a neonatal gnotobiotic piglet model.	<b>L.J. Saif</b> , A.N. Vlasova, S. Kandasamy, K. Chattha, G. Rajashekara, A. Kumar, A. Rauf, L. Shao, D. Fischer, H. Huang, S. Neal; Department of Veterinary Preventive Medicine, Food Animal Health Research Program, OARDC, The Ohio State University, Wooster, OH, USA.
<b>9:30</b>		<b>Break</b>	
		<b>Presider: Laura Miller and Aradhya Gourapura</b>	
<b>10:00-10:30 Tues.</b>	<b>120</b>	Directed Evolution Of An Adeno-Associated Virus Library <i>In Vivo</i> Pig Airway.	<b>J. Zabner</b> , Chief of Pulmonary and Critical Care, University of Iowa Hospitals & Clinics, Iowa City, IA.
<b>10:30-11:00</b>	<b>121</b>	Evaluating the metagenome of nasal samples from cattle with bovine respiratory disease complex (BRDC)	<b>T.G. McDanel</b> , L. Kuehn, J. Keele; US Meat Animal Research Center, Clay Center, NE, USA.
<b>11:00</b>	<b>122</b>	Evaluation of changes in VapA-specific IgG and IgG subclasses over time to identify foals with <i>Rhodococcus equi</i> pneumonia	<b>M.G. Sanz</b> , A. Oliveira Ferreira, A. Page, D. Horohov; University of Kentucky, Lexington, KY, USA.
<b>11:15 Tues.</b>	<b>123</b>	The effect of passively-acquired antibodies on <i>Lawsonia intracellularis</i> infection and immunity in the horse	<b>A.E. Page</b> <sup>1</sup> , H. Stills, Jr. <sup>2</sup> , D. Horohov <sup>1</sup> ; <sup>1</sup> Dept. of Veterinary Science, University of Kentucky, Lexington, KY, USA, <sup>2</sup> Dept. of Microbiology, Immunology, and Molecular Genetics, University of Kentucky, Lexington, KY, USA.
<b>11:45 to</b>	<b>12:30</b>	<b>Business Meeting, Dedication, New Members Introduction, and Graduate Student Competition Awards Presentations</b>	

# PATHOBIOLOGY OF ENTERIC AND FOODBORNE PATHOGENS

Los Angeles/Miami Room - 5th Floor

Section Leader: Radhey S. Kaushik and Weiping Zhang

Time	No.	Title	Authors
		<b>Presiders: Radhey S. Kaushik and Weiping Zhang</b>	
<b>8:00 Mon.</b>	<b>125</b>	Most Prevalent Poultry-associated Salmonella SeroTypes differ in their susceptibility to widely used carcass sanitizer, chlorine	<b>D.H. Shah</b> , N.C. Paul; Department of Veterinary Microbiology and Pathology, Washington State University, Pullman, WA, USA.
<b>8:15</b>	<b>126</b>	A randomized trial to assess whether enrofloxacin metaphylaxis for bovine respiratory disease affects fecal shedding of Salmonella and Campylobacter in feedlot cattle	<b>A.B. Smith</b> <sup>1</sup> , D.G. Renter <sup>1</sup> , N. Cernicchiaro <sup>1</sup> , X. Shi <sup>1</sup> , J.S. Nickell <sup>2</sup> , D.J. Keil <sup>2</sup> , T. Nagaraja <sup>1</sup> ; <sup>1</sup> College of Veterinary Medicine, Kansas State University, Manhattan, KS, USA, <sup>2</sup> Bayer Healthcare LLC, Shawnee, KS, USA
<b>8:30</b>	<b>127</b>	Characterization of a Putative Hemolysin of Campylobacter jejuni for Potential Use in Vaccination of Poultry.	<b>A. Armstrong</b> , M. Anderson, B. Law; University of Arizona, Tucson, AZ, USA.
<b>8:45-9:30 Mon. Keynote</b>	<b>128</b>	<b>Pathobiology of Enteric &amp; Foodborne Pathogens Keynote:</b> Plasmids of enteric bacterial pathogens: past, present, and future challenges	<b>T.J. Johnson</b> ; Veterinary and Biomedical Sciences, University of Minnesota, Saint Paul, MN, USA.
<b>9:30</b>		<b>Break and Table Top Exhibits – Foyer</b>	
<b>9:45</b>	<b>129</b>	Pooling of immunomagnetic separation beads does not affect sensitivity of detection of seven serogroups of Shiga toxin-producing <i>Escherichia coli</i> in cattle feces	<b>L.W. Noll</b> , W.C. Baumgartner, P. Belagola Shridhar, C.A. Cull, D.M. Dewsbury, X. Shi, N. Cernicchiaro, D.G. Renter, T. Nagaraja; Diagnostic Medicine and Pathobiology, Kansas State University, Manhattan, KS, USA.
<b>10:00 Mon.</b>	<b>130</b>	Transcriptional profiling of <i>Salmonella</i> Enteritidis strains identifies genes consistently highly expressed in biologically relevant microenvironments	<b>K. Chiok</b> , D.H. Shah; Department of Veterinary Microbiology and Pathology, Washington State University, Pullman, WA, USA.
<b>10:15</b>	<b>131</b>	Identification and characterization of immune-modulatory CpG motifs of <i>Salmonella</i>	<b>J.R. Elder</b> , D.H. Shah; Department of Veterinary Microbiology and Pathology, Washington State University, Pullman, WA, USA.
<b>10:30</b>	<b>132</b>	Diversity and distribution of a novel swine dysentery pathogen <i>Brachyspira hampsonii</i>	<b>N. Mirajkar</b> , A. Bekele, Y. Chander, C. Gebhart; Veterinary and Biomedical Sciences, University of Minnesota, Saint Paul, MN, USA.
<b>10:45 Mon.</b>	<b>133</b>	The humoral immune response of pigs and horses against the vaccine of Lawsonia intracellularis	Y. Nakamura <sup>1</sup> , D. Miyayama <sup>1</sup> , R. Uemura <sup>1</sup> , Y. Sasaki <sup>2</sup> , H. Niwa <sup>3</sup> , T. Higuchi <sup>4</sup> , <b>M. Sueyoshi</b> <sup>5</sup> ; <sup>1</sup> Dept. of Veterinary Science, University of Miyazaki, Miyazaki, Japan, <sup>2</sup> Org. for Promotion of Tenure-track, University of Miyazaki, Miyazaki, Japan, <sup>3</sup> Equine Research Institute, The Japan Racing Association, Tochigi, Japan, <sup>4</sup> Hidaka Agricultural Mutual Relief Association, Hokkaido, Japan, <sup>5</sup> The Center for Animal Disease Control, University of Miyazaki, Miyazaki, Japan.

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## PATHOBIOLOGY OF ENTERIC AND FOODBORNE PATHOGENS

Los Angeles/Miami Room - 5th Floor

Section Leader: Radhey S. Kaushik and Weiping Zhang

Time	No.	Title	Authors
11:00 Mon.	134	Temperature-dependent conjugative gene transfer in <i>Campylobacter jejuni</i>	<b>D. Ardeshta</b> , B. Gillespie, J. Lin, X. Zeng; Animal Science, University of Tennessee, Knoxville, TN, USA.
11:15	135	Determination of the minimum infectious dose of porcine epidemic diarrhea virus in neonatal and weaned pigs	<b>J.T. Thomas</b> , Q. Chen, P.C. Gauger, D. Madson, E.R. Burrough, D.R. Magstadt, H. Salzbrenner, M.W. Welch, L.G. Gimenez-Lirola, K.J. Yoon, J. Zhang; Department of Veterinary Diagnostic and Production Animal Medicine, Iowa State University, Ames, IA, USA.
11:30		<b>Lunch Break</b>	
4:30 to	5:00	<b>Break and Table Top Exhibits – Foyer</b>	
5:00 to	6:30	<b>Poster Session II Grand Ballroom Salon III - 7th floor</b>	

## RESPIRATORY DISEASES

Indiana/Iowa Room 6th Floor

Section Leaders: Amelia Woolums and Christopher Chase

Time	No.	Title	Authors
		<b>Presiders: Amelia Woolums and Chris Chase</b>	
8:00 Mon.	137	Effect of pretreatment on detection of PRRSV in oral fluid by qRT-PCR assay	<b>A. Holmes</b> , S. Abate, P. Gauger, W. Gonzalez, K. Yoon, C. Wang, J. Zimmerman; Veterinary Diagnostics and Production Animal Medicine, Iowa State University, Ames, IA, USA.
8:15	138	Vaccination mitigates the negative impact PRRSV infection has on the pharmacokinetics of ceftiofur crystalline free acid in pigs.	<b>J.W. Sparks</b> <sup>1</sup> , D.N. Day <sup>1</sup> , L.A. Karriker <sup>1</sup> , L.W. Wulf <sup>1</sup> , J. Zhang <sup>1</sup> , J.L. Bates <sup>1</sup> , R. Gehring <sup>2</sup> , J.F. Coetzee <sup>1</sup> ; <sup>1</sup> Veterinary Diagnostic and Production Animal Medicine, Iowa State University, Ames, IA, USA, <sup>2</sup> Anatomy and Physiology, Kansas State University, Manhattan, KS, USA
8:30	139	Genetic diversity analysis of genotype 2 porcine reproductive and respiratory syndrome viruses emerging in recent years in China	<b>L. Zhou</b> , X. Yang, Y. Tian, S. Yin, G. Geng, X. Ge, X. Guo, H. Yang; Veterinary Medicine, China Agricultural University, Beijing, China.
8:45 Mon.	140	<i>In vivo</i> targeting of porcine reproductive and respiratory syndrome virus antigen through porcine DC-SIGN to dendritic cells elicits antigen-specific CD4 T cell immunity in pigs	<b>S. Subramaniam</b> , P. Piñeyro, D. Tian, C. Overend, D.M. Yugo, S.R. Matzinger, A.J. Rogers, M.R. Haac, Q. Cao, C.L. Heffron, N. Catanzaro, S.P. Kenney, Y.-W. Huang, T. Opriessnig, X.-J. Meng; Virginia Tech, Blacksburg, VA, USA.

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**RESPIRATORY DISEASES**  
**Indiana/Iowa Room 6th Floor**

**Section Leaders: Amelia Woolums and Christopher Chase**

<b>Time</b>	<b>No.</b>	<b>Title</b>	<b>Authors</b>
<b>9:00 Mon.</b>	<b>141</b>	Neuraminidase inhibiting (NI) antibodies induced in pigs by experimental influenza A virus vaccines	<b>M.R. Sandbulte</b> <sup>1</sup> , G. Nordholm <sup>2</sup> , A. Vincent <sup>2</sup> ; <sup>1</sup> Veterinary Microbiology & Preventive Medicine, Iowa State University, Ames, IA, USA, <sup>2</sup> Virus and Prion Research Unit, National Animal Disease Center, USDA-ARS, Ames, IA, USA.
<b>9:15</b>	<b>142</b>	Influenza transmission within coordinated swine production systems	<b>J.F. Lowe</b> <sup>1</sup> , B. Kaplan <sup>2</sup> , R. Webby <sup>2</sup> ; <sup>1</sup> Veterinary Clinical Medicine, University of Illinois, Albers, IL, USA, <sup>2</sup> Infectious Disease, St. Jude's Children's Research Hospital, Memphis, TN, USA.
<b>9:30</b>		<b>Break and Table Top Exhibits – Foyer</b>	
<b>10:00</b>	<b>143</b>	Co-circulation of two distinct genetic and antigenic lineages of proposed influenza D virus in cattle	<b>E.A. Collin</b> <sup>1</sup> , Z. Sheng <sup>2</sup> , Y. Lang <sup>3</sup> , W. Ma <sup>3</sup> , B. Hause <sup>3</sup> , F. Li <sup>4</sup> ; <sup>1</sup> Newport Laboratories Inc / SDSU, Worthington, MN, USA, <sup>2</sup> Columbia University, New York, NY, USA, <sup>3</sup> Kansas State University, Manhattan, KS, USA, <sup>4</sup> Dept Veterinary and Biomedical Sciences, South Dakota State University, Brookings, SD, USA.
<b>10:15 Mon.</b>	<b>144</b>	An RSV fusion inhibitor is an effective treatment for bovine respiratory syncytial virus infection of calves	<b>L. Gershwin</b> <sup>1</sup> , R. Jordan <sup>2</sup> , M. Anderson <sup>1</sup> , H. McEligot <sup>1</sup> , N. Behrens <sup>1</sup> , M. Perron <sup>2</sup> , T. Cihlar <sup>2</sup> , S. Lewis <sup>2</sup> , E. Eisenberg <sup>2</sup> , H. Hui <sup>2</sup> , A. Carey <sup>2</sup> , R. Strickley <sup>2</sup> , R. Mackman <sup>2</sup> ; <sup>1</sup> Pathology, Microbiology, & Immunology, University of California, Davis, Davis, CA, USA, <sup>2</sup> Gilead Sciences, Inc., Foster City, CA, USA.
<b>10:30</b>	<b>145</b>	Improving case definitions for BRD treatment with an algorithm based analysis of lung auscultation	<b>E. Grimmer</b> ; College of Veterinary Medicine, University of Illinois, Urbana, IL, USA.
<b>10:45</b>	<b>146</b>	Identification of pens at high risk for BRD with an algorithm based analysis of thoracic auscultation at post arrival processing	<b>J. Lowe</b> <sup>1</sup> , K. Brattain <sup>2</sup> , G. Taylor <sup>2</sup> , T. Noffsinger <sup>3</sup> , W. Taylor <sup>3</sup> , D. French <sup>1</sup> , B. Aldridge <sup>1</sup> ; <sup>1</sup> Veterinary Clinical Medicine, University of Illinois, Urbana, IL, USA, <sup>2</sup> Geissler Corp. LLC, Plymouth, MN, USA, <sup>3</sup> Production Animal Consultation, LLC, Oakley, KS, USA.
<b>11:00 Mon.</b>	<b>147</b>	Acute phase proteins in naturally occurring respiratory disease of feedlot cattle: a novel approach to diagnosis.	<b>I. Idote</b> <sup>1</sup> , M. Heller <sup>2</sup> , B. Vander Ley <sup>1</sup> ; <sup>1</sup> University of Missouri, College of Veterinary Medicine, Columbia, MO, USA, <sup>2</sup> University of California Davis, Davis, CA, USA.

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**RESPIRATORY DISEASES**  
**Indiana/Iowa Room 6th Floor**

**Section Leaders: Amelia Woolums and Christopher Chase**

<b>Time</b>	<b>No.</b>	<b>Title</b>	<b>Authors</b>
<b>11:15 Mon.</b>	<b>148</b>	Cytokine profiles from shipping through sickness and recovery in cattle either mass-medicated with gamithromycin or sham-treated	<b>C.G. Chitko-McKown<sup>1</sup></b> , K.D. DeDonder <sup>2</sup> , G.L. Bennett <sup>1</sup> , M.D. Apley <sup>2</sup> , G.P. Harhay <sup>1</sup> , L.A. Kuehn <sup>1</sup> , B.J. White <sup>2</sup> , R.L. Larson <sup>2</sup> , S.F. Capik <sup>2</sup> , B.V. Lubbers <sup>2</sup> , A.M. Workman <sup>1</sup> ; <sup>1</sup> U.S. Meat Animal Research Center, Clay Center, NE, USA, <sup>2</sup> College of Veterinary Medicine, Kansas State University, Manhattan, KS, USA.
<b>11:30</b>		<b>Lunch Break</b>	
<b>1:30 to 2:30</b>		<b>ACVM-CE Mini-Symposium - Avenue Ballroom, 4th Floor</b>	
<b>2:45</b>		<b>Break and Table Top Exhibits – Foyer</b>	
		<b>Presiders: Amelia Woolums and Chris Chase</b>	
<b>3:00 Mon.</b>	<b>149</b>	Identification and characterization of bovine rhinitis viruses in bovine respiratory disease clinical specimens	<b>B.M. Hause</b> , R.A. Hesse, G. Anderson; Veterinary Diagnostic Laboratory and Department of Diagnostic Medicine and Pathobiology, Kansas State University, Manhattan, KS, USA.
<b>3:15 Mon.</b>	<b>150</b>	Characterization of Biofilm Formation by <i>Pasteurella multocida</i>	<b>B.L. Petruzzi<sup>1</sup></b> , R.E. Briggs <sup>2</sup> , C. De Castro <sup>3</sup> , A. Molinaro <sup>3</sup> , T. Inzana <sup>1</sup> ; <sup>1</sup> Biomedical and Veterinary Sciences, Virginia Maryland Regional College of Veterinary Medicine, Blacksburg, VA, USA, <sup>2</sup> Respiratory Diseases of Livestock Unit, US Department of Agriculture, Ames, IA, USA, <sup>3</sup> Dipartimento di Scienze Chimiche, Universita di Napoli Federico II, Via Cintia, Italy.
<b>3:30</b>	<b>151</b>	Septic pleuropneumonia in 41 horses (2000 - 2014)	<b>S. Taylor</b> ; Veterinary Clinical Sciences, Purdue University, West Lafayette, IN, USA.
<b>3:45-4:30 Mon. Keynote</b>	<b>152</b>	<b>Respiratory Diseases Keynote:</b> Regulation of interferon-gamma gene expression in foals and its relationship to susceptibility to <i>Rhodococcus equi</i> .	<b>D.W. Horohov</b> ; Veterinary Science, University of Kentucky, Lexington, KY, USA.
<b>4:30 to 5:00</b>		<b>Break and Table Top Exhibits – Foyer</b>	
<b>5:00 to 6:30</b>		<b>Poster Session II Grand Ballroom Salon III - 7th floor</b>	

# VECTOR-BORNE AND PARASITIC DISEASES

Denver/Houston - 5th Floor

Section Leader: Roman Ganta and Roger W. (Bill) Stich

Time	No.	Title	Authors
8:00 Mon.	153	Real-time PCR Assay Validation for Detecting Rickettsia rickettsii infections in dogs and ticks	<b>A. DS Nair</b> , N. Bhoi, R. Raghavan, R.R. Ganta; Diagnostic Medicine and Pathobiology, Kansas State University, Manhattan, KS, USA.
8:15	154	Molecular prevalence of Theileria spp. in ruminants from nine provinces of China	Y. Yang <sup>1</sup> , Y. Mao <sup>2</sup> , P. Kelly <sup>3</sup> , Z. Yang <sup>2</sup> , L. Luan <sup>1</sup> , J. Zhang <sup>1</sup> , J. Li <sup>1</sup> , H.S. El-Mahallawy <sup>1</sup> , <b>C. Wang<sup>1</sup></b> ; <sup>1</sup> College of Veterinary Medicine, Yangzhou University, Yangzhou, China, <sup>2</sup> College of Animal Science and Technology, Yangzhou University, Yangzhou, China, <sup>3</sup> College of Veterinary Medicine, Ross University, P.O. Box 308, Saint Kitts and Nevis
8:30	155	Portable insulated isothermal RT-PCR (iiRT-PCR) assay for sensitive and specific detection of bluetongue virus	<b>A. Ambagala<sup>1</sup></b> , S. Pahari <sup>1</sup> , M. Fisher <sup>1</sup> , T. Furukawa-Stoffer <sup>1</sup> , B. Agboton <sup>1</sup> , J. Pasick <sup>2</sup> , E.N. Ostlund <sup>3</sup> , D.J. Johnson <sup>3</sup> , P.A. Lee <sup>4</sup> , H.T. Wang <sup>4</sup> , O. Lung <sup>1</sup> ; <sup>1</sup> National Centres for Animal Disease, Canadian Food Inspection Agency, Lethbridge, AB, Canada, <sup>2</sup> National Centres for Animal Disease, Canadian Food Inspection Agency, Winnipeg, MB, Canada, <sup>3</sup> Equine and Ovine Viruses Section, National Veterinary Services Laboratories, Ames, IA, USA, <sup>4</sup> Department of Research and Development, GeneReach USA, Lexington, MA, USA.
8:45	156	Surveillance of ecto- and endoparasitism in northern Mississippi canine shelter populations	<b>U. Donnett</b> , J. Shivley, K. Woodruff; Department of Clinical Sciences, Mississippi State University College of Veterinary Medicine, Starkville, MS, USA.
9:00	157	Evaluation of Dermacentor variabilis for the incidence of pathogens causing diseases in animals and humans	<b>N. Bhoi<sup>1</sup></b> , A.D. Nair <sup>2</sup> , G.A. Anderson <sup>1</sup> , R.R. Ganta <sup>2</sup> , R.K. Raghavan <sup>1</sup> ; <sup>1</sup> Department of Diagnostic Medicine/Pathobiology and Kansas State Veterinary Diagnostic Laboratory, Kansas State University, Manhattan, KS, USA, <sup>2</sup> Department of Diagnostic Medicine/Pathobiology, Kansas State University, Manhattan, KS, USA
9:15 Mon.	158	Investigation into horn fly burden susceptibility in Holstein heifers	<b>B. Blair<sup>1</sup></b> , C. Ramirez <sup>2</sup> , B. Aldridge <sup>2</sup> , J. Lowe <sup>2</sup> , D. French <sup>2</sup> ; <sup>1</sup> Year 3 Student College of Veterinary Medicine, Integrated Food Animal Medicine Systems, University of Illinois, Urbana, IL, USA, <sup>2</sup> Integrated Food Animal Medicine Systems, University of Illinois, Urbana, IL, USA
9:30		<b>Break and Table Top Exhibits – Foyer</b>	

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## VECTOR-BORNE AND PARASITIC DISEASES

Denver/Houston - 5th Floor

Section Leader: Roman Ganta and Roger W. (Bill) Stich

Time	No.	Title	Authors
10:00-10:45 Mon. Keynote	159	<b>Vector-Borne &amp; Parasitic Diseases</b> <b>Keynote:</b> Bartonellosis: One Health Perspectives on an Emerging Infectious Disease.	<b>E.B. Breitschwerdt</b> ; Dept of Clinical Sciences and the Center for Comparative Medicine & Translational Research, CVM, North Carolina State University, Raleigh, NC, USA.
10:45	160	Rickettsia felis in China	J. Zhang <sup>1</sup> , G. Lu <sup>1</sup> , P. Kelly <sup>2</sup> , Z. Zhang <sup>3</sup> , L. Wei <sup>1</sup> , D. Yu <sup>4</sup> , <b>C. Wang<sup>1</sup></b> ; <sup>1</sup> College of Veterinary Medicine, Yangzhou University, Yangzhou, China, <sup>2</sup> College of Veterinary Medicine, Ross University, Basseterre, Saint Kitts and Nevis, <sup>3</sup> Subei People's Hospital, Yangzhou, China, <sup>4</sup> College of Medicine, Yangzhou University, Yangzhou, China
11:00-11:30		Open	
11:30		<b>Lunch Break</b>	
4:30 to 5:00	5:00	<b>Break and Table Top Exhibits – Foyer</b>	
5:00 to	6:30	<b>Poster Session II Grand Ballroom Salon III - 7th floor</b>	

## VIRAL PATHOGENESIS

Los Angeles/Miami Rooms - 5th Floor

Section Leader: Kyoung-Jin Yoon

Time	No.	Title	Authors
11:30		<b>Lunch Break</b>	
1:30 Mon.	161	A unique mechanism of protein-directed trans-activation of ribosomal frameshifting in arteriviruses	<b>Y. Li<sup>1</sup></b> , E.E. Treffers <sup>2</sup> , S. Naphthine <sup>3</sup> , A. Tas <sup>2</sup> , L. zhu <sup>1</sup> , B.L. Mark <sup>4</sup> , P.A. van Veelen <sup>2</sup> , A.E. Firth <sup>3</sup> , I. Brierley <sup>3</sup> , E.J. Snijder <sup>2</sup> , Y. Fang <sup>1</sup> ; <sup>1</sup> Diagnostic Medicine / Pathobiology, Kansas State university, Manhattan, KS, USA, <sup>2</sup> Molecular Virology Laboratory, Leiden university Medical center, Leiden, Netherlands, <sup>3</sup> Department of Pathology, University of Cambridge, Cambridge, UK, <sup>4</sup> Department of Microbiology, University of Manitoba, Winnipeg, MB, Canada.
1:45	162	Regulatory role of the SAP-like motif of PRRSV nsp1 $\beta$ protein for innate immune response	<b>M. Han</b> , D. Yoo; Pathobiology, University of Illinois at Urbana-Champaign, Urbana, IL, USA.
2:00	163	The PRRSV-mediated inhibition of IFN $\alpha$ production by pig alveolar macrophages occurs at the post-transcriptional level via the activation of eIF-2 $\alpha$	<b>W.-Y. Chen</b> , G. Calzada-Nova, W. Schnitzlein, F.A. Zuckermann; Pathobiology, University of Illinois, Urbana, IL, USA.

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**VIRAL PATHOGENESIS**  
**Los Angeles/Miami Rooms - 5th Floor**  
**Section Leader: Kyoung-Jin Yoon**

Time	No.	Title	Authors
<b>2:15 Mon.</b>	<b>164</b>	Development of a synthetic porcine reproductive and respiratory syndrome virus strain that confers broader cross-protection	<b>H. Vu<sup>1</sup></b> , F. Ma <sup>1</sup> , W. Laegreid <sup>2</sup> , A. Pattmaik <sup>1</sup> , F. Osorio <sup>1</sup> ; <sup>1</sup> School of Veterinary medicine and Biomedical Sciences, University of Nebraska-Lincoln, Lincoln, NE, USA, <sup>2</sup> Veterinary Sciences, University of Wyoming, Laramie, WY, USA.
<b>2:30</b>	<b>165</b>	Characterization of entry events during bile acid-mediated porcine enteric calicivirus replication	<b>V. Shivanna</b> , Y. Kim, K.-O. Chang; Kansas State University, Manhattan, KS, USA.
<b>2:45</b>		<b>Break and Table Top Exhibits – Foyer</b>	
<b>3:00</b>	<b>166</b>	The spike protein furin cleavage site in feline coronavirus is not the sole determinant of conversion to feline infectious peritonitis virus	<b>Y.-C. Juan</b> , D. Gao, E. Chowdhury, K. Rahman, B. Kaltenboeck; Pathobiology, Auburn University, Auburn, AL, USA.
<b>3:15</b>	<b>167</b>	Assessment of viremia and tissue distribution of porcine epidemic diarrhea virus in weaned pigs after experimental infection	<b>M. Bhandari</b> , D. Madson, L. Bower, E. Burrough, D. Magstadt, P. Arruda, D. Sun, H. Hoang, G. Stevenson, K.J. Yoon; College of Veterinary Medicine, Iowa State University, Ames, IA, USA.
<b>3:30</b>	<b>168</b>	Sequencing analysis of recently outbreaked porcine epidemic diarrhea virus in vietnam	<b>N. Park<sup>1</sup></b> , D. Song <sup>2</sup> , M. Hong <sup>1</sup> , W. Na <sup>1</sup> , M. Yeom <sup>1</sup> ; <sup>1</sup> Viral Infectious Disease Research Center, KRIBB, Daejeon, Korea, Republic of, <sup>2</sup> Viral Infectious Disease Research Center, KRIBB, daejeon, Korea, Republic of
<b>3:45</b>	<b>169</b>	Pathogenesis of US porcine deltacoronavirus strains FD22 and FD100 in gnotobiotic pigs.	<b>K. Jung</b> , B. Eyerly, H. Hu, Z. Lu, L.J. Saif; Food Animal Health Research Program, The Ohio State University, Wooster, OH, USA.
<b>4:00 Mon.</b>	<b>170</b>	Development and validation of an indirect porcine deltacoronavirus (PDCoV) anti-IgG ELISA based on the S1 portion of the spike protein and confirmation that PDCoV infection in U.S. pigs is low and has been present since 2010	<b>A. Thachil<sup>1</sup></b> , C.-T. Xiao <sup>1</sup> , P.F. Gerber <sup>2</sup> , P.G. Halbur <sup>1</sup> , Y.W. Huang <sup>3</sup> , T. Opriessnig <sup>2</sup> ; <sup>1</sup> Iowa State University, Ames, IA, USA, <sup>2</sup> The Roslin Institute, University of Edinburgh, Midlothian, UK, <sup>3</sup> Zhejiang University, Hangzhou, China.
<b>4:15</b>		Open	
<b>4:30 to 5:00</b>		<b>Break and Table Top Exhibits – Foyer</b>	
<b>5:00 to 6:30</b>		<b>Poster Session II Grand Ballroom Salon III - 7th floor</b>	

**VIRAL PATHOGENESIS**  
**Los Angeles/Miami Rooms - 5th Floor**  
**Section Leader: Kyoung-Jin Yoon**

Time	No.	Title	Authors
<b>8:00 Tues</b>	<b>172</b>	African swine fever virus (ASFV) rp30 ELISA detects antibody in serum and/or oral fluid specimens	<b>L.G. Giménez-Lirola</b> <sup>1</sup> , L. Mur <sup>2</sup> , B. Rivera <sup>2</sup> , C. Wang <sup>3</sup> , C. Goodell <sup>4</sup> , R.B. Rowland <sup>5</sup> , D.L. Harris <sup>6</sup> , C. Gallardo <sup>7</sup> , M. Arias <sup>7</sup> , J. Sánchez-Vizcaíno <sup>2</sup> , J.J. Zimmerman <sup>1</sup> ; <sup>1</sup> Department of Veterinary Diagnostic and Production Animal Medicine, Iowa State University, Ames, IA, USA, <sup>2</sup> VISAVET Center and Animal Health, University Complutense of Madrid, Madrid, Spain, <sup>3</sup> Department of Veterinary Diagnostic and Production Animal Medicine & Department of Statistics, Iowa State University, Ames, IA, USA, <sup>4</sup> Department of Veterinary Diagnostic and Production Animal Medicine, IDEXX Laboratories, Westbrook, ME, USA, <sup>5</sup> Department of Diagnostic Medicine and Pathobiology, Kansas State University, Manhattan, KS, USA, <sup>6</sup> Harrisvaccines, Inc, Ames, IA, USA, <sup>7</sup> CISA-INIA, Madrid, Spain.
<b>8:15</b>	<b>173</b>	Acute infection with bovine viral diarrhea virus causes depletion of WC1+ $\gamma\delta$ T cells in lymphoid tissues in beef calves	<b>R.A. Palomares</b> <sup>1</sup> , K. Sakamoto <sup>2</sup> , H. Walz <sup>3</sup> , K. Brock <sup>4</sup> , D.J. Hurley <sup>1</sup> ; <sup>1</sup> Population Health, University of Georgia, College of Veterinary Medicine, Athens, GA, USA, <sup>2</sup> Pathology, University of Georgia, College of Veterinary Medicine, Athens, GA, USA, <sup>3</sup> Pathobiology, Auburn University, College of Veterinary Medicine, Auburn, AL, USA, <sup>4</sup> Pathobiology, Auburn University, College of Veterinary Medicine, Auburn, AL, USA
<b>8:30</b>	<b>174</b>	The bovine immunodeficiency virus Rev protein: characterization of the multimerization domain using the bimolecular fluorescence complementation (BiFC) technology	<b>C. Marchand</b> , A. Gomez Corredor, D. Archambault; Sciences Biologiques, Université du Québec à Montréal, Montréal, QC, Canada.
<b>8:45 Tues.</b>	<b>175</b>	Efficacy of M2e-based vaccine in murine, avian and swine models	<b>C.W. Lee</b> <sup>1</sup> , K.-I. Kang <sup>1</sup> , M. Elaish <sup>1</sup> , J.M. Ngunjiri <sup>1</sup> , H. Jang <sup>1</sup> , A. Ali <sup>2</sup> , J. Hiremath <sup>1</sup> , S. Dhakal <sup>1</sup> , M. Xia <sup>3</sup> , X. Jiang <sup>3</sup> , R. Gourapura <sup>1</sup> ; <sup>1</sup> Food Animal Health Research Program, The Ohio State University, Wooster, OH, USA, <sup>2</sup> Poultry Diseases, Beni-Suef University, Beni-Suef, Egypt, <sup>3</sup> Division of Infectious Diseases, Cincinnati Children's Hospital Medical Center, Cincinnati, OH, USA

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**VIRAL PATHOGENESIS**  
**Los Angeles/Miami Rooms - 5th Floor**  
**Section Leader: Kyoung-Jin Yoon**

Time	No.	Title	Authors
<b>9:00 Tues.</b>	<b>176</b>	Potential of endogenously produced and exogenously administered interferon as adjuvant for influenza vaccine in animals	<b>J.M. Ngunjiri</b> <sup>1</sup> , H. Jang <sup>2</sup> , C.-W. Lee <sup>2</sup> ; <sup>1</sup> Food Animal Health Research Program, The Ohio State University, WOOSTER, OH, USA, <sup>2</sup> Food Animal Health Research Program, and Department of Veterinary Preventive Medicine, The Ohio State University, WOOSTER, OH, USA.
<b>9:15</b>	<b>177</b>	Poly I:C adjuvanted inactivated swine influenza vaccine induces heterologous protective immunity in pigs	M. Thomas <sup>1</sup> , Z. Wang <sup>2</sup> , C.C. Sreenivasan <sup>1</sup> , B.M. Hause <sup>3</sup> , R.J. Gourapura <sup>4</sup> , F. Li <sup>1</sup> , D.H. Francis <sup>1</sup> , R.S. Kaushik <sup>1</sup> , <b>M. Khatri</b> <sup>4</sup> ; <sup>1</sup> South Dakota State University, Brookings, SD, USA, <sup>2</sup> Food Animal Health, South Dakota State University, Brookings, SD, USA, <sup>3</sup> Kansas State University, Manhattan, KS, USA, <sup>4</sup> Food Animal Health, Ohio State University, Wooster, OH, USA.
<b>9:30</b>		<b>Break</b>	
<b>10:00- 10:45 Keynote</b>	<b>178</b>	<b>Distinguished Veterinary Microbiologist Keynote: Viral Pathogenesis: Lessons Learned from Newcastle Disease Virus.</b>	<b>S.K. Samal</b> ; VA-MD Regional College of Veterinary Medicine, University of Maryland, College Park, MD, USA.
<b>10:45 Tues.</b>	<b>179</b>	Rescue and characterization of a new reassortant influenza virus strain H5N1	<b>A.D. Zaberezhny</b> <sup>1</sup> , T.V. Grebennikova <sup>2</sup> , G.K. Vorkunova <sup>2</sup> , A.G. Yuzhakov <sup>2</sup> , L.V. Kostina <sup>2</sup> , S.N. Norkina <sup>2</sup> , T.I. Aliper <sup>2</sup> , E.A. Nepoklonov <sup>3</sup> , D.K. Lvov <sup>2</sup> ; <sup>1</sup> Y.R.Kovalenko All-Russian Institute of Experimental Veterinary Medicine, Moscow, Russian Federation, <sup>2</sup> D.I.Ivanovski Virology Institute, Moscow, Russian Federation, <sup>3</sup> Federal Service for Veterinary and Phytosanitary Surveillance, Moscow, Russian Federation.
<b>11:00</b>	<b>180</b>	Naturally truncated NS gene of H3N8 equine influenza virus attenuates the virulence of the A/Puerto Rico/8/34 virus	<b>W. Na</b> , S. Yoon, T. Lee, M. Hong, M. Yeom, N. Park, D. Song; Viral Infectious Disease Research Center, KRIBB, Daejeon, Korea, Republic of.
<b>11:15 Tues.</b>	<b>181</b>	Role of Ebola virus matrix protein in regulating cellular innate immune response	<b>H. Sooryanarain</b> , S. Elankumaran; Department of Biomedical Sciences and Pathobiology, Virginia Tech, Blacksburg, VA, USA.
<b>11:45 to</b>	<b>12:30</b>	<b>Business Meeting, Dedication, New Members Introduction, and Graduate Student Competition Awards Presentations</b>	

# **POSTER ABSTRACTS**

## Bacterial Pathogenesis Posters

001P

Seroprevalence of *Yersinia enterocolitica* in different mammalian hosts at a nonhuman primate research facility

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*Yersinia enterocolitica* is a Gram negative zoonotic member of the family Enterobacteriaceae. An outbreak of *Y. enterocolitica* led to the deaths of 46 captive African Green monkeys (AGM) on the island of St. Kitts. In order to gain a better understanding of the epizootiology of the bacterium, a multi-species study was undertaken to investigate the prevalence and seroprevalence of *Y. enterocolitica* in various animals that reside in the farm or use the grounds for feeding. Blood was collected from 105 AGMs, 12 mice, 10 rats, 3 mongooses, 12 feral cats and 2 dogs. Plasma was tested utilizing the RecombLine *Yersinia* IgG 2.0 strip western blot kits to investigate the seroprevalence. The AGM samples revealed a seroprevalence of 89.5% for *Yersinia* spp. and a seroprevalence of 59% for *Y. enterocolitica*. Interestingly, animals that were housed in cages where at least one fatality occurred during the outbreak showed similar seroprevalence as those housed in non-affected cages. However, the animals in the non-affected and seropositive cages were significantly older than the animals in affected (presenting at least one fatality during outbreak) and seropositive cages. In conclusion these findings show a very high seroprevalence of *Y. enterocolitica*, indicating that during and after the outbreak, the agent was able to disseminate with high success through the captive AGM population, and that age is a risk factor for the development of clinical yersiniosis. Additionally, the seroprevalence of *Y. enterocolitica* in feral cats, dogs, mongooses, rats, and mice was 25%, 100%, 33%, 10%, and 16%, respectively. Potential introduction of the pathogen by humans, wild AGM, other vertebrates or invertebrates remain a possibility that requires further study. Future research in wild animal populations, as well as in the local human population and environment, should help us understand the epidemiology of this enigmatic pathogen in the Caribbean.

002P

Phylogenetic analysis of *Dermatophilus congolensis* isolated from naturally infected cattle in Abeokuta and Ilorin, Nigeria.

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*Dermatophilus congolensis*, the aetiological agent of dermatophilosis, is a pleomorphic, Gram-positive actinomycete which infects animals and humans. Oftentimes there is wrong diagnosis of the infection in animals because of the close resemblance of the organism with other members of the family *Actinomycetaceae*. In this study, molecular tools were applied for the confirmatory diagnosis of dermatophilosis from suspected isolates of *D. congolensis* obtained from naturally infected cattle in Nigeria. DNA extraction from 54 suspected pure colonies of *D. congolensis* was carried out using the QIAamp<sup>®</sup> DNA Mini extraction kit. PCR targeted at the 16S rRNA gene was employed for the confirmation of *D. congolensis* using 5'-ACATGCAAGTCGAACGATGA-3' and 5'-ACGCTCGCACCTACGTATT-3' as forward and reverse primers respectively. Positive amplicons were then sequenced directly using Big Dye Terminator Cycle Sequencing Kit with the forward primer and AmpliTaq-FS DNA Polymerase. Nucleotide sequences were aligned using BioEdit software while the phylogenetic analysis was carried out using MEGA 5.2 software. The aligned nucleotide sequences of 10 positive *D. congolensis* had between 94% to 99% homology with the sequences of *D. congolensis* satellite DNA in GenBank. Phylogenetic analysis revealed that *D. congolensis*, though closely related to *Nocardia brasiliensis* (NR 074743.01) and *Streptomyces* sp. (JN 400114.1), belong to different genera. It was also revealed that only 2 (D362 and D363) of the 10 *D. congolensis* clustered with previously listed strains available in GenBank, while the remaining 8 (D367, D368, D364, D373, D374, D356, D357 and D360) clustered out separately. In conclusion, molecular tools employed in the study were able to confirm the identity of the suspected isolates as *D. congolensis*. Additionally, the 2 strains of *D. congolensis* obtained from the study are closely linked to the previously listed strains available in GenBank, while the remaining 8 may be different strains of *D. congolensis* not yet listed in GenBank.

003P

MALDI-TOF as a novel detection method for *Clostridium difficile* toxins

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**Purpose:** Toxigenic *Clostridium difficile* is an important cause of enterocolitis in equine, porcine and canine veterinary species and humans, and can be identified by the presence of one or both toxins: Toxin A, an enterotoxin, and toxin B, a cytotoxin. Isolation of *C. difficile* from fecal samples is not enough to diagnose the cause of diarrhea, as non-toxigenic isolates may be present in the feces of clinically normal animals. Detection of toxins A and/or B is important to differentiate a disease-causing toxigenic strain from a non-toxigenic strain, and this is most commonly accomplished through use of a cytotoxicity assay (CTA) or an enzyme linked immunosorbent assay (ELISA). Because matrix assisted laser desorption ionization - time-of-flight mass spectrometry (MALDI-TOF) is becoming a common method for bacterial identification and accurately identifies *C. difficile* isolates, a MALDI-TOF-based proteolytic assay was evaluated in this study as a novel means for *C. difficile* toxin detection.

**Methods:** We used MALDI-TOF to analyze proteolytic digests of purified, commercially available *C. difficile* toxins and six *C. difficile* isolates in liquid culture, including three ATCC isolates and three clinical isolate from the archives of the Indiana Animal Disease Diagnostic Laboratory, with known toxin status based on CTA and ELISA.

**Results:** The peptide masses detected from purified *C. difficile* toxins were compared with expected masses generated with the EXPASY online peptide mass tool. Three peptide masses for toxin A and six peptide masses for toxin B were detected by MALDI-TOF. Detection of toxins in culture was less successful due to low concentrations.

**Conclusions:** MALDI-TOF shows promise as a novel means for *C. difficile* toxin detection, but further studies are warranted to optimize detection of toxins from culture.

## Bacterial Pathogenesis Posters

004P

Serological investigations of *Lawsonia intracellularis* in horses on breeding farms in Japan

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**Purpose:** Recently, equine proliferative enteropathy (EPE), caused by the intracellular bacterium *Lawsonia intracellularis* (Li), has been increasing in horse breeding farms in Japan. In this study, we performed serological epidemiologic studies of EPE in a thoroughbred-producing area in Hokkaido, Japan.

**Methods:** In Total, 478 horses on 110 farms were investigated. Serum samples collected in 1992-2013 were tested by an IFA method to detect Li-antibodies. In study 1, forty four EPE suspected horses in 38 farms which occurred in 2010-2013, 147 non-symptoms exhibiting horses in the same herds were examined. In study 2, horses exhibiting no symptoms (0-year-old 6 horses, 2012, 1-year-old 57 horses, 2013) were examined around an epidemic period in one farm that EPE had occurred in. In study 3, sixty one horses in 6 farms without EPE were examined. In study 4, one hundred horse (one-year-old) storage serum specimens from 64 farms from 1992 to 2013 were examined.

**Results:** In study 1, the Li-antibody positive rate of affected horses and non-symptoms exhibiting horses in the same herds were 93% (41/44) and 73% (108/147), respectively. In study 2, the Li-antibody positive rate was 0% (0/6) before the epidemic, 17% (1/6) during the epidemic and 100% (6/6) after the epidemic period in 2012, respectively. In 2013, the rate was 23% (13/57) before the epidemic, 63% (35/56) during the epidemic and 80% (45/56) after the epidemic period, respectively. In study 3, the Li-antibody positive rate was 17% (1/6) of the farms and 1.6% (1/61) of the horses. In study 4, the Li-antibody positive rate was 18% (18/100) in all of tested horses. In particular, the serum samples in 1992 were Li-antibody positive.

**Conclusions:** This study revealed that the asymptomatic horses in the EPE outbreak farms were also infected with Li at a high rate. The Li-antibody of the infected horses remained partly in the next year, and the re-infection was suggested as a possibility. Furthermore, spread around the farms was suggested because the Li-antibody after the epidemic was more positive in rate than it was before the epidemic. The horses infected with Li in 1992 were detected in Japan from the results of a retrograded investigation.

## Companion Animal Epidemiology Posters

005P

Effect of dog body condition score on lipoprotein cholesterol and triglyceride concentrations; effect of recommended fasting duration on sample concentrations

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**Purpose:** The objectives of this study were to survey clinics' guidance about recommended fasting duration (FD) prior to lipoprotein analysis, and to characterize lipoprotein cholesterol and triglyceride concentrations in obese and overweight dogs.

**Methods:** A dataset of dog characteristics was created from medical records of dogs that had serum samples submitted for lipoprotein analysis. The dataset included medical records of 1,665 dogs from 77 breeds in 360 clinics between January 2012 and December 2013. On the basis of the 5-point body condition score (BCS) scale, dogs were categorized as thin (BCS = 1), underweight, ideal, overweight or obese (BCS = 5). A phone survey was also conducted to obtain the information about the clinics' FD. Fasting duration was categorized into three groups; less than 8 hours, 8 to 11.9 hours and 12 hours or more. A two-level linear mixed-effects model was applied using a clinic at level 2 and a dog at level 1.

**Results:** Just over 50% (183) of the clinics said they recommended fasting for 12 hours or more. Dogs in clinics with FD 12 hours or more had lower chylomicron (CM) triglyceride concentrations than those in clinics with FD less than 11.9 hours ( $P = 0.07$ ). However, there were no differences between any of the FD groups for very low density lipoprotein (VLDL), low density lipoprotein (LDL) and high density lipoprotein (HDL) cholesterol and triglyceride concentrations ( $P > 0.26$ ). Mean ( $\pm$  SEM) BCS and age at sampling, for the 1,521 dogs in 350 clinics that had BCS information, were  $3.7 (\pm 0.02)$  and  $8.5 (\pm 0.09)$  years old, respectively. Obese and overweight dogs had higher VLDL cholesterol concentrations than ideal dogs ( $P < 0.05$ ), and also tended to have higher HDL cholesterol concentrations ( $P = 0.08$ ). However, no difference was found between dog BCS groups for cholesterol concentrations in LDL ( $P = 0.31$ ). Additionally, obese and overweight dogs had higher VLDL and HDL triglyceride concentrations than ideal dogs ( $P < 0.05$ ), but no such difference was found for LDL triglyceride concentrations ( $P = 0.10$ ).

**Conclusions:** Obese and overweight dogs were characterized by high VLDL and HDL cholesterol and triglyceride concentrations.

006P

Risk factors associated with obese or overweight dogs visited at Japanese private veterinary clinics

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**Purpose:** The objective of this study was to quantify risk factors and interactions between the risk factors associated with dogs being obese or overweight.

**Methods:** The dataset comprised medical records of 5,827 dogs from 103 breeds with body condition score (BCS) and dog characteristics, recorded in 942 clinics between 2006 and 2013. On the basis of the 5-point BCS scale, dogs were categorized as thin (BCS = 1), underweight, ideal, overweight or obese (BCS = 5). Dogs were also classified into five body size groups based on their breed. Multilevel logistic regression models were applied for the probability of dogs being obese or overweight, by using a clinic at level 2 and a dog at level 1.

**Results:** Mean values ( $\pm$  SEM) of BCS and age at sampling were  $3.6 (\pm 0.01)$  and  $8.4 (\pm 0.05)$  years old, respectively. Of the 5,827 dogs, 13.7% were obese and 37.9% were overweight. Risk factors for obese dogs were increased age, female dogs and medium sized dogs, whereas those for overweight dogs were increased age, female dogs and neutered dogs ( $P 0.09$ ). In small and toy sized dogs, neutered dogs were more likely to be obese or overweight than intact dogs, whereas in medium sized dogs, neutered dogs were less likely to be obese than intact dogs ( $P < 0.05$ ).

**Conclusions:** The effects of age and neutering on the probabilities of dogs being obese or overweight differed depending on breed body size. Therefore, by taking age, body size and neuter status into account, veterinarians can better advise owners about the maintenance of their dogs in ideal body condition.

## Companion Animal Epidemiology Posters

007P

Multidrug resistant infections in dogs in a veterinary teaching hospital: risk factors for fecal carriage.

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Abstract Not Available

008P

Extended-spectrum beta-lactamase producing enterobacteriaceae recovered from healthy dogs.

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Objective: *bla*<sub>CMY-2</sub> and *bla*<sub>CTX-M</sub> encode production of extended-spectrum beta-lactamases that confer resistance to important antimicrobial agents used in both humans and animals. Their epidemiology has been described for livestock and clinical samples from companion animals. However, little is known about their frequency and distribution in the GI flora of healthy companion animals. The aim of this study is to estimate the prevalence of these resistance genes in the fecal flora of presumably-healthy dogs providing samples for routine parasitology screening at the OSU Veterinary Medical Center.

Methods: Fecal samples from canine fecal submissions (n=212) for parasite screening between January and April 2013 were enriched in a nutrient broth containing 2 ug/ml cefotaxime. This broth was subsequently inoculated onto MacConkey Agar with 8 ug/ml of cefoxitin, 4 ug/ml of cefepime, or 2 ug/ml of meropenem, to identify the *bla*<sub>CMY</sub>, *bla*<sub>CTX-M</sub>, and carbapenemase phenotypes. Genotypes were confirmed using PCR.

Results: Among the 12 isolates expressing a phenotype consistent with *bla*<sub>CTX-M</sub>, 9 were confirmed to belong to CTX-M Group 1. Our estimated prevalence of *bla*<sub>CTX-M</sub> in canine fecal samples is thus 4.25% (95% CI 1.51% – 6.98%). Among the 75 isolates with a phenotype consistent with *bla*<sub>CMY</sub>, 30 were confirmed *bla*<sub>CMY-2</sub>. Our estimated prevalence of *bla*<sub>CMY-2</sub> in canine fecal samples is thus 14.15% (95% CI 9.42% – 18.88%).

Discussion: Our results suggest that canine companion animals have a similar fecal prevalence of *bla*<sub>CTX-M</sub> as livestock, while *bla*<sub>CMY-2</sub> prevalence appears to be lower than has been reported in livestock populations. Additional investigation to identify subsets of dogs at greatest risk for colonization would be beneficial to determining risk factors such as historical antibiotic use, surgical implant, ICU patient, exposure to livestock, or other high risk populations.

009P

Exposure factors associated with side effects in dogs after treatment with Chinese herbs

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Purpose: The use of traditional Chinese veterinary medicine (TCVM) for treatment of sick companion animals has increased in the last ten years. In a survey conducted by the Chi Institute in Florida, the frequency of companion animals identified with side effects (e.g., gastrointestinal (GI) disorders, skin reactions, elevated liver enzymes) after treatment with Chinese herbs was 63/15,000 or 0.42% (unpublished data). Although the frequency of side effects is considered low, there is interest among TCVM practitioners to elucidate risk factors that may predispose dogs to development of side effects. The objective of this study was to identify risk factors for side effects in dogs after treatment with Chinese herbs.

Methods: An on-line survey was prepared and tested, and later distributed via e-mail to 7,000 veterinary practitioners in USA, Australia, and European countries in July 2014. This investigation was designed as a case-control study. Investigated exposure factors included age, gender, breed, spay/neuter status, body weight, presenting complaint(s) at admission, herbal treatment(s) used and duration of treatment, as well as other medication(s) and diet(s) used during treatment.

Results: Preliminary results using 32 case dogs and 32 control dogs have revealed the following findings. The most common side effect among cases was GI disorders (18/32 or 56%). Among cases and controls, the most common complaint at admission was pain (26/64 or 41%). Using univariable logistic regression, the variables for gender and multiple complaints at admission had values of  $p \leq 0.10$ .

Conclusions: Results and discussion of the final epidemiologic analysis will be presented at the conference.

010P

Creation of a bioarchive to the prevalent brucella strains in Egypt

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In this study different animal species, including cattle, buffaloes, sheep, and goats were investigated. A total number of 59 brucella isolates from 11 governorates were deeply investigated together with six reference Brucella strains. The obtained results showed that 55 field isolates were typed as *Brucella melitensis* biovar 3. The remaining isolates were identified as *B. abortus* biovar 1 (n=2) and *B. suis* biovar 1 (n=2). High degree of similarity among the 55 cultures of *Brucella melitensis* biovar 3 in this

investigation did not permit the detection of quantitative differences at the sub-biovar level. Antibiotic resistogram typing was performed on the 55 *Brucella melitensis* biovar 3 isolates for strain discrimination and as a trial for tracing back of their origin. A total number of 21 antibiotics at certain 29 sub therapeutic concentrations were used. Of the 21 antibiotics used, 10 proved to be powerful differentiating tools often giving qualitative variations. Results showed that 25 out of the 55 isolates of *Brucella melitensis* biovar 3 were successfully differentiated from each other. Twenty three isolates of *Br. melitensis* biovar 3 from cows included nine strains as indicated by differences in correlation coefficients of antibiotic resistogram patterns. The obtained results indicated that antibiotic resistogram typing could be used as a powerful tool for epidemiological trace back of field brucella strains. Evaluation of the genetic heterogeneity of a panel of 17 *Brucella* spp. isolates recovered from domestic ruminants (cattle, buffalo, sheep and goat) was carried out using PCR, MLVA and sequencing. Thirteen isolates were identified as *B. melitensis* belonging to nine different MLVA profiles, yielding a high discriminatory power of the technique (allelic diversity = 0.8677). The remaining isolates were identified as *B. abortus* (n=2) and *B. suis* biovar 1 (n=2). This is the first report of the isolation of *B. suis* from cattle in



## Companion Animal Epidemiology Posters

(010P continued)

Egypt. Results of the present study suggest a high genetic diversity of the *B. melitensis* strains circulating among domestic ruminants in Egypt and a potential role of livestock as reservoirs of other zoonotic *Brucella* species as *B. suis biovar 1* and *B. abortus*.

## Ecology and Management of Foodborne Agents Posters

011P

Specificity analysis and detection software evaluation of PathoProof™ Mastitis PCR Assay

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PathoProof PCR™ (Finnzymes, Espoo, Finland) Mastitis PCR Complete 12 Assay detects the 11 most common bovine mastitis pathogens as well as the beta-lactamase gene responsible for penicillin resistance. The assay uses real-time PCR to screen suspected milk samples for mastitis-causing bacteria yielding a result in four hours whereas conventional culture can take up to 48 hours. The assay's specificity was evaluated by performing PCR on 17 organisms previously identified using MALDI TOF (Matrix-Assisted Laser Desorption Ionization Time of Flight). The Applied Biosystems® 7500 Real-Time PCR System was used for the testing. Results were analyzed with the Norden® software and numerous samples that were expected to be negative were called positive. Upon closer examination in the "component" view using the instrument's SDS (Sequence Detection System) software it was determined that the amplification curves generated from these samples did not exhibit the typical sigmoidal shape and were therefore being called positive erroneously. Subsequently all analysis was performed with the system's SDS software, with baseline and threshold set independently for each target. The 11 organisms targeted by the assay's primers and probes were appropriately identified. The remaining 6 organisms, which are not identified by the Mastitis PCR assay, yielded negative results.

012P

Association between temperament and *E. coli* O157:H7 shedding in Brahman calves.

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Cattle are asymptomatic carriers of enterohemorrhagic *E. coli* O157:H7, a cause of food borne disease. Multiple factors, such as diet and transportation stress influence *E. coli* O157:H7 shedding. We investigated whether temperament and weaning stress affect *E. coli* O157:H7 prevalence in Brahman calves (6 to 7 months of age; 45 heifers; 33 bulls). Based on temperament score (average of pen score and exit velocity) calm, intermediate and temperamental animals respectively comprised 30, 55 and 15 % of the sampled calves. Rectal grab fecal samples and blood samples were collected at weaning (d0) and 4 days post-weaning (d4). Selective media and immunomagnetic bead separation were used for detecting *E. coli* O157:H7 in feces. Serum cortisol concentration was determined by RIA. Antimicrobial susceptibility of isolates was accessed by automatic microdilution method. Seventeen and 25 calves shed *E. coli* O157:H7 on d0 and d4, respectively. Twelve calves shed *E. coli* O157:H7 on both days. The majority of the isolates were susceptible to the antimicrobials included in the National Antimicrobial Resistance Monitoring System testing of Gram negative organisms with the exception of the 55% of isolates that were resistant to sulfisoxazole. The serum cortisol concentration of the calves positive for *E. coli* O157:H7 (16.9±2.6 ng/mL) on both days did not differ ( $P > 0.10$ ) from that of calves that were negative on one or both of the sampling days. As described previously, there was a strong association of increased cortisol concentrations (females 21.5±1.7 ng/mL, males 14.6±2.0 ng/mL) with temperament and sex ( $P < 0.05$ ). However, there was no association between *E. coli* O157:H7 prevalence and elevated serum cortisol associated with a more excitable temperament at weaning and post-weaning in Brahman calves. Future assessment of bovine intestinal microbiota composition will help to understand the effect of temperament-associated elevated serum cortisol concentrations on the microbial community of Brahman calves at weaning and post-weaning.

013P

Stress and non-typhoidal *Salmonella enterica*: quantifying the impact of stocking density in dairy cattle.

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Purpose: Livestock are important reservoirs of non-typhoidal *Salmonella enterica* (NTS). However, no research to date has examined the association among stress, stocking density and NTS shedding around calving. The objective of this study was to quantitatively determine the impact of stress caused by higher stocking density at the feed bunk on the NTS shedding through the periparturient period in dairy cattle. Methods: This longitudinal study included 120 cows from a commercial dairy farm in Ohio. Block randomization was used to assign dry cows into one of four groups with different stocking density conditions at the feed bunk (1.33 versus 0.67 headlocks/cow). Study groups included: A) overstocking from 60 to 1, B) 60 to 20, C) 20 to 1, and D) understocked from 60 to 1 d prior to calving. In total, 360 fecal and blood samples were collected at 3 time points relative to calving (-60, -15, and +7 d). Cultures of fecal samples were used to determine the NTS prevalence, and qPCR was used to estimate NTS concentration. Indicators of stress were measured using serum concentrations of  $\beta$ -hydroxybutyrate (BHBA) and non-esterified fatty acids (NEFA), and fecal concentrations of cortisol metabolites. NTS fecal concentration was used as response in a regression analysis. NEFA, BHBA, age, treatment group, sample, and treatment\*sample were considered for inclusion in the statistical model. Results: Preliminary results demonstrate that a substantial proportion of cows began shedding NTS closer to the calving day. Overall, 75.0% (90/120) and 80.6% (93/114) of cows were culture positive at 60 and 15 d prior to calving, respectively. The prevalence of NTS at -60 and -15 d for each group was, respectively: A) 66.7% (20/30), 73.3% (22/30), B) 70% (21/30), 81.8% (18/22), C) 73.3% (22/30), 72.2% (21/29), and D) 90.0% (27/30), 96.7% (29/30). The association between fecal concentrations of *Salmonella* and stress measurements will be presented. Conclusions: This study offers an opportunity to understand how stress associated with stocking density influences the ecology of NTS in dairy farms. Data generated from this study could lead to specific farm management strategies to decrease the prevalence of NTS on dairy farms.

## Ecology and Management of Foodborne Agents Posters

014P

Changes in the frequency of reported human illnesses associated with *Salmonella* subtypes recovered from Michigan dairy farms in either 2000-2001 or 2009.

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**Purpose:** Non-typhoidal *Salmonella enterica* is a global cause of foodborne illnesses. Temporal changes in the distribution of subtypes in livestock populations may have important impacts on human health. This group reported within-farm changes in the population of subtypes of *Salmonella* on Michigan dairy farms that were sampled in both time frames. Briefly, the overall distribution of multi-locus sequence types was significantly different between time frames ( $p < 0.05$ ), and only two of 31 PFGE patterns identified in 2000-2001 were again recovered from the same set of farms in 2009. The objective of this research was to determine the changes in the yearly frequency (2000 through 2012) of reported human illnesses in Michigan associated with the PFGE patterns recovered on Michigan dairy farms in either 2000-2001 or 2009.

**Methods:** PFGE patterns of 11 serotypes recovered from dairy farms were obtained from the Michigan Department of Community Health (MDCH). Human-associated patterns indistinguishable from dairy farm patterns were identified, and the yearly frequency was tabulated.

**Results:** Forty-two percent (4,705/11,326) of the human cases were associated with one of the 11 serotypes that were recovered from Michigan dairy farms in either 2000-2001 or 2009. Of 48 PFGE patterns representing 11 serotypes recovered on dairy farms, 12 (25%) matched at least one of the 716 patterns of the same serotypes associated with a human infection. However, only 387/11,326 (3.4%) of salmonellosis cases reported in Michigan between 2000 and 2012 were associated with the dairy farm subtypes. For the subtypes associated with human illnesses, the frequency of human illnesses decreased for patterns recovered from dairy farms in only in 2000-2001, and increased for patterns only recovered in 2009.

**Conclusions:** Most *Salmonella* subtypes found on dairy farms were infrequent causes of illness in humans. Nonetheless, there were opposing trends in the yearly frequency of reported human illnesses for subtypes recovered in 2000-2001 and 2009.

015P

Distribution and characteristics of shipments of mail-order hatchlings containing outbreak-associated *Salmonella* subtypes

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**Purpose:** Non-typhoidal *Salmonella enterica* (NTS) is a worldwide cause of illness in people. The frequency of outbreaks of zoonotic salmonellosis associated with live poultry contact has increased over the previous 20 years. In 2014, a multiple-serotype outbreak of zoonotic salmonellosis was traced to contact with live poultry, some of which were purchased at a national chain of farm stores. The objective of the study was to analyze the characteristics of shipments arriving at the national chain farm stores and positive outbreak-associated NTS strains.

**Methods:** This study used cultures of chick pads from shipment boxes of hatchling poultry, data on the shipment characteristics and breeds, and USPS tracking codes. Multiple bedding culture samples were collected from shipment boxes that had sections containing different breeds. Bedding underwent standard culture and enrichment procedures for *Salmonella*. Recovered isolates underwent serotyping, pulsed-field gel electrophoresis, and antimicrobial resistance testing.

**Results:** Overall, 17% (61/361) of bedding cultures were positive, and 30% (72/237) of shipment boxes had at least one positive bedding culture. The proportion of shipment boxes was not significantly different across hatcheries; however, shipment boxes containing the tetra tint breed were significantly more likely to be positive for any strain of NTS relative to other breeds. Strains with PFGE patterns indistinguishable from concurrent multistate outbreaks of salmonellosis in humans were recovered.

**Conclusions:** This study provides epidemiologic information on the distribution and sources of NTS strains causing human outbreaks. These data are useful to focus efforts on control of *Salmonella* at the hatchery level; however additional research is necessary to design effective interventions.

016P

Wild birds as Potential Vectors for *Salmonella* on Ohio Dairies

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**Purpose:** Wild birds are known to be reservoirs for *Salmonella* spp. Previous studies have found that *Salmonella* is found sporadically in the intestinal flora of wild birds. Birds are attracted to farms due to the presence of feedstuffs and their close contact with livestock provides the potential for pathogen transmission. Enteric pathogens such as *Salmonella* can colonize a wide variety of species and thus could be transmitted between livestock and wild birds. The symptoms of salmonellosis are potentially severe and can impact animal health as well as profitability of the farm, and so prevention is important.

The goal of this study was to determine if wild birds play a role in the transmission of *Salmonella* as either a mechanical vector by transmitting *Salmonella* on their feet or feathers or as a biological vector through their excrement on Ohio dairy farms.

**Methods:** External and cloacal swabs were taken from 346 live wild birds captured with mist nets near three free stall barns in Ohio.

Environmental bovine fecal samples were also collected from the barns at the dairy. Swabs were placed in nutrient broth and incubated for 24 hours at 37°C and then moved to Rappaport Vassiliadis (RV) broth. The RV broth was incubated for 24 hours at 42°C and then streaked for isolation onto XLT4 agar. Identity of bacteria as *Salmonella* was confirmed using standard biochemicals. The cow fecal samples were screened in a similar manner.

**Results:** Of the wild birds sampled, *Salmonella* was isolated from one cloacal swab from a house sparrow. Although the dairy cows had a high prevalence of *Salmonella*, this did not directly correlate with the prevalence of *Salmonella* in wild birds that were within 500 yards of the barn.

**Conclusions:** Based on our findings, wild birds likely do not play an important role in the transmission of *Salmonella* on Ohio dairy farms.

## Ecology and Management of Foodborne Agents Posters

017P

Application of MALDI-TOF MS for the identification and characterization of AMR bacteria from wildlife associated with concentrated animal feeding operations

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**Purpose:** The prevalence of antimicrobial resistant (AMR) bacteria in concentrated animal feeding operations (CAFOs) may be effectively reduced by interventions which limit contact of wildlife with livestock. As such, it is imperative to characterize and monitor the exchange of AMR bacteria between wildlife and CAFOs, ultimately leading to improved interventions that target the specific problem wildlife vector(s). Thus, methods for profiling of AMR bacteria are needed to assess their transmission between wildlife and livestock. Here, matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) was used to profile AMR bacteria from CAFOs.

**Methods:** MALDI-TOF MS of ethanol/formic acid extracted bacteria was performed using the Bruker Ultraflex II TOF/TOF. Microbial profiling was accomplished using the Bruker MALDI Biotyper RTC software (Ver. 3.1.). A total of 1,177 presumptive AMR *Escherichia coli* (ciprofloxacin and cefotaxime resistant), *Staphylococcus* spp. (cefoxitin resistant), and *Enterococcus* spp. (erythromycin resistant) isolates from wildlife, feed, and water associated with CAFOs were analyzed.

**Results:** MALDI-TOF MS identified 849 of the 1177 isolates, which represented 17 different genera and 48 different species. Mass spectra were obtained for an additional 296 isolates, but could not be matched to reference spectra. 71.5% and 90.2% of the isolates presumptively identified as *E. coli* (n = 302) and *Enterococcus* spp. (n = 505) using culture based methods, respectively, were confirmed as these species. Only 3.2% of presumptive *Staphylococcus* isolates (n = 370) were confirmed as *Staphylococcus* spp. Additional detailed analyses of the mass spectra identified numerous features useful for subtyping applications, including differentiation of AMR phenotypes.

## Epidemiology and Animal Health Economics Posters

018P

Nasal, skin and tonsillar carriage of methicillin-resistant and zinc-resistant *Staphylococcus aureus* in piglets fed diets supplemented with zinc and chlortetracycline

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Zinc (Zn) is often supplemented at elevated concentrations (2,000-3,000 ppm) in swine diets to prevent enteric infections and promote growth. Studies from Denmark have suggested a genetic linkage and a phenotypic association between Zn resistance, encoded by *czrC*, and methicillin-resistance, encoded by *mecA*, and tetracycline resistance in *S. aureus*. Such an association has not been reported in the U.S. swine population. We conducted a study to evaluate the association of in-feed Zn and chlortetracycline (CTC) supplementations on the prevalence of MRSA in pigs. The study consisted of 240 weaned piglets, housed in 48 pens (5 piglets/pen), randomly assigned to six treatments in a 2×2×2 incomplete factorial design. Treatment factors included diets with normal (30 ppm) or high (2,500 ppm) concentration of Zn, with and without CTC at low (5 mg/kg BW) or high (22 mg/kg BW) levels, and the combinations of high Zn with the two CTC doses. Nasal, skin, and tonsillar swabs were collected from all piglets on days 0, 21, and 42. The samples were inoculated on to MRSA CHROMagar and presumptive MRSA colonies were confirmed by genus (*Staph*) and species (*nuc*) specific PCR. The isolates were then tested for *mecA* and *czrC* genes by PCR. Statistical analyses for binary endpoints were carried out using STATA (v.12.1). The prevalence of *mecA*-positive MRSA was 42.8% (308/720), 37.2% (268/720), and 42.6% (307/720) in nasal, skin, and tonsillar samples, respectively. The prevalence of *czrC*-positive MRSA was 20% (144/720), 21.1% (152/720), and 14.3% (103/720) in nasal, skin, and tonsillar swabs, respectively. The prevalence of *mecA* and *czrC* genes was not affected by Zn or CTC supplementation ( $P > 0.05$ ). The sampling day and sampling day by treatment interaction had a significant effect on the prevalence of *mecA* and *czrC*-positive MRSA in all three collection sites ( $P < 0.001$ ). The occurrence of *czrC* gene was strongly associated with *mecA*-positive MRSA isolates from all three collection sites ( $P < 0.0001$ ). Studies to genotypically and phenotypically characterize MRSA isolates are in progress.

019P

High lifetime and reproductive performance of sows in Southern EU commercial herds can be predicted by high numbers of pigs born alive at parity 1

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**Purpose:** Our objectives were 1) to compare reproductive performance across parity and lifetime performance in sow groups categorized by the number of pigs born alive (PBA) in parity 1, and 2) to examine the factors associated with high PBA in parity 1.

**Methods:** We analyzed 476,816 parity records and 109,373 lifetime records of females entered into 125 herds in 2005-2007. Sows were categorized into four groups based on the 10th, 50th and 90th percentiles of PBA in parity 1, as follows: 7 pigs or fewer, 8-11 pigs, 12-14 pigs and 15 pigs or more. Sows were also classified into three gilt age at first-mating (AFM) groups based on the upper and lower 25th percentiles of AFM, as follows: 229 days or earlier, 230-277 days and 278 days or later. Generalized linear models were applied to the data.

**Results:** Sows having 15 pigs or more in parity 1 had 0.5-1.8 higher PBA in any subsequent parity than the other 3 groups, and they also had a higher farrowing rate in parities 1 and 2 than sows having 7 pigs or fewer ( $P < 0.05$ ). However, there were no differences between the sow PBA groups for farrowing rate in parity 3 or higher, or for weaning-to-first-mating interval in any parity ( $P > 0.05$ ). For lifetime performance, there were two-way interactions between the sow PBA groups and the AFM groups ( $P < 0.05$ ). Sows having 15 pigs or more in parity 1 had 22.9-26.2 higher lifetime PBA and 0.6-0.9 higher parity at removal than sows having 7 pigs or fewer ( $P < 0.05$ ), regardless of AFM. Also, for sows having 14 pigs or fewer in parity 1, sows with AFM 229 days or earlier had 3.4-3.8 higher lifetime PBA and 0.4-0.7 higher parity at removal than sows with AFM 278 days or later ( $P < 0.05$ ). For PBA in parity 1, there was a two-way interaction between birth month groups and AFM ( $P < 0.05$ ); the PBA in parity 1 females born between January and June increased by 0.3-0.5 pigs, when AFM increased from 200 to 320 days old ( $P < 0.05$ ).

## Epidemiology and Animal Health Economics Posters

(019P continued)

However, the same rise in AFM only increased the PBA of females born from July to September by 0.1 pigs ( $P < 0.05$ ), and had no effect on the PBA of females born from October to December ( $P = 0.37$ ).

Conclusions: High PBA at parity 1 be used to predict a sow's reproductive and lifetime performance. Also, AFM of less than 278 days can be a target in the studied population.

020P

Abortion occurrence, repeatability and factors associated with abortions in female pigs in Southern EU commercial herds

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Purpose: Our objectives were 1) to define abortion occurrences including abortion rate and abortion risk, 2) to examine factors associated with abortions and 3) to determine repeatability of abortions in commercial herds.

Methods: We analyzed 122,935 lifetime records and 692,887 service records of females entered into 125 herds in 2008-2010. Annualized abortion rate (%) was defined as the number of abortion records divided by the sum of the reproductive herd life days x 365 days x 100. Also, abortion risks per service (%) were defined as the number of abortion records divided by the number of service records x 100. Generalized linear models with random intercept were applied to the data.

Results: Annualized abortion rate ( $\pm$  SEM) and abortion risk per service were  $4.1 \pm 0.06$  and  $1.5 \pm 0.01\%$ , respectively. Risk factors associated with an increased abortion risk per service were high or low parity, more stillborn piglets in the previous farrowing, prolonged weaning-to-first-mating interval (WMI), services in July and August and re-serviced sows ( $P < 0.05$ ). When the number of stillborn piglets increased from 0 to 3 pigs, the probability of a sow aborting increased by 0.2% ( $P < 0.05$ ). Also, sows with WMI 7 days or later had 0.2% higher abortion risk per service than sows with WMI 0-6 days ( $P < 0.05$ ). Females mated in July and August had 0.4-0.5% higher abortion risk per service than those mated in March and April ( $P < 0.05$ ). There was a two-way interaction between parity and the number of services for abortion risk per service ( $P = 0.07$ ); for first-serviced females, parity 0 and parity 6 or higher females had 0.2% higher abortion risk per service than parity 1 sows ( $P < 0.05$ ). However, there were no such differences in re-serviced females ( $P > 0.05$ ). Of the females aborted once, 2.7-8.6% experienced a second abortion in any parity. The repeatability for abortions was estimated to be 23.5-23.9%.

Conclusions: Females at risk of having an abortion are characterized as being in high or low parity, mated in summer and having more stillborn piglets, having prolonged WMI or re-services. These females should be monitored closely.

021P

Interactions between climatic factors, parity and total pigs born for occurrences and numbers of stillborn piglets during hot and humid or cold seasons in breeding herds

**R. Iida**, Y. Koketsu; School of Agriculture, Meiji University, Kawasaki, Japan.

Purpose: Our objective was to quantify interactions between climatic factors and production factors for the occurrence of a sow having stillborn piglets (SBP), and for the number of SBP, during either hot and humid or cold seasons.

Methods: We analyzed 161,299 parity records from 101 herds; all the sows farrowed either between June and September (hot and humid season) or between December and March (cold season). The climate data were obtained from 21 weather stations close to the herds. For each parity record, average daily maximum (Tmax) and minimum temperatures (Tmin) for the farrowing date and the previous day were coordinated with the performance data. Multilevel logistic regression and Poisson regression models with random intercept and slopes were conducted for whether or not a sow had SBP in a given litter, and for the number of SBP by sows that farrowed one or more SBP, respectively.

Results: The occurrences ( $\pm$  SEM) of sows having SBP during the hot and humid season and during the cold season were  $49.6 \pm 0.17\%$  and  $48.8 \pm 0.18\%$ , respectively. For sows that farrowed one or more SBP, mean SBP was  $2.0 \pm 0.01$  pigs in the two seasons. Mean values (ranges) of Tmax in the hot and humid season and Tmin in the cold season were  $28.4$  ( $13.6$  to  $39.8$ ) °C and  $2.1$  ( $-13.2$  to  $17.6$ ) °C, respectively. In the hot and humid season, the occurrences of SBP by parity 3 or higher sows increased by 2.1-2.7% as Tmax increased from 25 to 30 °C ( $P < 0.05$ ). However, Tmax was not associated with the occurrences of parity 1-2 sows having SBP ( $P = 0.73$ ). Also, a sow exposed to 30 °C had 0.04 more SBP than one exposed to 25 °C ( $P < 0.05$ ). In the cold season, the occurrences of sows in any parity group having SBP increased by 0.9-2.2% as Tmin decreased from 5 to 0 °C ( $P < 0.05$ ). Also, for sows that farrowed 16 pigs (90th percentile) the occurrence of sows having SBP and the number of SBP increased by 2.7% and 0.09 pigs, respectively as Tmin decreased from 5 to 0 °C ( $P < 0.05$ ). However, there were no such associations with Tmin for sows that farrowed only 8 pigs (10th percentile;  $P \geq 0.89$ ).

Conclusions: Tmax in summer and Tmin in winter appear to be critical for the occurrence of SBP and for the number of SBP, taking into account parity and total pigs born.

022P

Mastitis and pregnancy loss in first lactation dairy cows

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The purpose of this study is to investigate the association between mastitis alone (or in combination with other factors) and pregnancy loss in dairy cows 6 weeks before and after first pregnancy diagnosis, particularly during their first lactation period.

The study population is a dairy farm with ~ 500 Holstein lactating cows. Records from a total of 1,018 first-lactation, pregnant cows during 2006-2014 were considered for inclusion. Pregnancy diagnosis in study cows was performed by ultrasound on day 32 or 33 after artificial insemination. Confirmation of the pregnancy was achieved by trans-rectal palpation 2 and 6 weeks after of pregnancy diagnosis. Logistic regression will be used to identify and quantify the relationship between mastitis and other factors and pregnancy loss in study cows.

A preliminary, descriptive analysis using a random sample of 100 records has revealed the following information. Twelve cows were diagnosed with pregnancy loss. A total of 1, 3, 2, and 2 cows were diagnosed with clinical mastitis during 8-14, 22-28, 29-35, and 36-42 days before pregnancy diagnosis, respectively. In addition, 2, 2, 2, 1, and 3 cows were diagnosed with clinical mastitis during 1-7, 8-14, 15-21, 22-28, 29-35, and 36-42 days after pregnancy diagnosis.

Final results on the association between mastitis alone (or in combination with other health, environmental, and management factors) and pregnancy loss in dairy cows will be presented at the conference.

## Epidemiology and Animal Health Economics Posters

023P

Heat stress related dairy cow mortality during heat waves and control periods in rural Southern Ontario from 2010-2012

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Heat stress is a physiological response to extreme environmental heat such as heat waves. Heat stress can result in mortality in dairy cows when extreme heat is both rapidly changing and has a long duration. As a result of climate change, heat waves, which are defined as 3 days of temperatures of 32°C or above, are an increasingly frequent extreme weather phenomenon in Southern Ontario. Heat waves are increasing the risk for on-farm dairy cow mortality in Southern Ontario. Heat stress indices (HSIs) are generally based on temperature and humidity and provide a relative measure of discomfort which can be used to predict increased risk of on-farm dairy cow mortality. In what follows, the heat stress distribution is predictable over space and can be presented by maps. Similarly, on-farm mortality is predictable and can be mapped.

Mortality records and farm locations for all farms registered in the CanWest Dairy Herd Improvement Program in Southern Ontario were retrieved for 3 heat waves and 6 three day control periods from 2010-2012. A random sample of controls (2:1) was taken from the data set to create a risk-based hybrid design. On-farm heat stress was estimated using data from 37 weather stations and subsequently interpolated across Southern Ontario by geostatistical kriging. A Poisson regression model was applied to assess the on-farm mortality in relation to varying levels of the HSI.

For every one unit increase in HSI the on-farm mortality rate across Southern Ontario increases by 1.03 times (CI95% (IRR)=(1.025,1.035);  $p \leq 0.001$ ). With a typical 8.6 unit increase in HSI from a control period to a heat wave, mortality rates are predicted to increase by 1.27 times. These findings will aid policymakers (e.g., local government) and farmers in making informed decisions about best practices for cooling and heat abatement strategies to improve farm animal welfare.

024P

HoBi-like pestiviruses persistently infected calves transmit the virus to calves, sheep, goats and pigs

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Similar to bovine viral diarrhoea viruses (BVDV), HoBi-like viruses can establish persistently infections in cattle. The purpose of this study was to determine if HoBi persistently infected (PI) calves could transmit the virus to other ruminants and swine. Ten calves, eight sheep, seven goats and ten pigs free of pestivirus infection were used. Animals from each species were divided into two experimental groups. One group (PI cohort or PIC) was directly exposed to HoBi PIs while the other group (inoculated IN) was inoculated with  $2.5 \times 10^5$  TCID<sub>50</sub> (tissue culture infecting dose) of two HoBi-like virus strains (HoBi\_D32/00 and Italy-1/10-1 strains). The PIC groups were composed of five calves, four sheep, four goats and five pigs housed by species and exposed to two HoBi PI calves (one PI harboring the strain HoBi\_D32/00 and one the strain Italy-1/10-1) for 30 minutes, twice a day, for seven consecutive days. Animals were monitored for pyrexia and signs of respiratory or enteric disease for 23 days post inoculation or 23 days from first exposure to PI calves. Nasal swab and blood samples were collected at days -2, 3, 6, 9, 11, 13 and 18 post-infection. RT-PCR was performed using primers specific for HoBi-like viruses. Pyrexia and viremia was observed in all calves on at least one time point. The average number of days with pyrexia was 2.3 for IN and 3.6 for PIC. One calf died at day 17 post-infection. Virus was detected in the buffy coat of this animal at time of death. Nasal discharge was observed in the four sheep in the IN and one at the PIC (one day average). One sheep in the IN presented fever at day 5 post-infection, and diarrhoea and ocular discharge for four days. Virus was detected in four and three sheep in the IN and PIC, respectively. Viremia was detected in a single IN goat, while pyrexia was observed in three goats for each group. The average number of days with pyrexia was 5.3 and 3.7 for IN and PIC. Virus was detected in two pigs in the IN and one in the PIC. In addition, two of the IN pigs and all of the PIC pigs developed pyrexia. While the most efficient transmission was observed with cattle, transmission was observed in all species tested. HoBi-like viruses therefore pose a potential threat to the ruminant and swine industries.

025P

Canadian Integrated Program for Antimicrobial Resistance Surveillance (CIPARS) broiler chicken farm surveillance: 2013 *Salmonella* results  
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CIPARS Broiler Chicken Farm Surveillance was initiated in 2013 in the 4 major Canadian poultry-producing provinces of British Columbia, Alberta, Ontario, and Québec. The objectives of the program are to: establish a national farm surveillance infrastructure; provide data on antimicrobial use and resistance; investigate associations between antimicrobial use and resistance; and provide data for human health risk assessments. Poultry veterinarians were enrolled and subsequently recruited representative flocks to participate in this voluntary program. Flocks were visited one time per year during the last week of grow (>30 days of age) for sample and data collection. Four pooled fecal samples (one sample per floor quadrant) consisting of at least 10 fecal droppings were collected from randomly selected barns and floors. A proportion of flocks were also visited at placement and two pooled barn surface environmental sponge swabs and three pooled chick pad meconium swabs were collected. All samples were cultured for *Salmonella* and quantitative antimicrobial susceptibility testing was performed using the Sensititre® Microbiology System (Trek Diagnostic Systems, Cleveland, OH, USA). The recovery rate of *Salmonella* varied between sampling time (placement vs. pre-harvest) and sampling type (environmental vs. fecal). Overall recovery was 45%. At placement recovery was 22% but when broken down by sampling type chick pads (30%) had higher recovery rates than environmental samples (10%). At pre-harvest, the recovery rate increased to 59%. The top 3 serovars overall were Kentucky, Enteritidis and Heidelberg. At placement, *S. Enteritidis* isolates were largely detected from chick pads (33%) with only 1 isolate detected from the environmental samples (11%). All of the *S. Enteritidis* isolates were susceptible to the antimicrobials tested. Ceftiofur resistance was detected in 24% of all *Salmonella* isolates. When broken down by sampling time ceftiofur resistance was slightly higher at placement (27%) compared to pre-harvest (23%). Isolates that were resistant to 4-5 classes of antimicrobials were detected only at pre-harvest. No isolates were resistant to >5 antimicrobial classes.

## Epidemiology and Animal Health Economics Posters

026P

Prevalence of a highly virulent *Campylobacter jejuni* clone associated with sheep abortion in feedlot cattle in the United States

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A highly pathogenic, tetracycline-resistant *C. jejuni* clone (clone SA) has emerged as the predominant *Campylobacter* species causing sheep abortion in the United States during the last decade. Clone SA is also a zoonotic pathogen transmitted mainly via raw milk consumption and causing gastroenteritis in humans. This study was conducted to determine the prevalence and distribution of clone SA on multiple cattle farms across the United States. A total of 3,184 fecal samples were collected from 66 different feedlot cattle herds in Iowa, Texas, Colorado, Missouri and Kansas, and cultured directly for *Campylobacter*. The overall prevalence rate of *Campylobacter* in cattle feces was 72% (2293/3184). A species-specific PCR identified majority of the isolates (82.6%, 1893/2293) as *C. jejuni*, and most of the rest was *C. coli* (89%, 356/400). Within *C. jejuni* 8.7% (164/1893) were initially identified as clone SA using a gene-specific PCR. Further confirmation by pulse-field gel electrophoresis (PFGE) using KpnI restriction enzyme revealed that 112 of these isolates as clone SA, giving an overall prevalence of rate of 5.9% (112/1893). The prevalence rate of clone SA varied by state: 1.8% (14/759) in Colorado, 2.1% (17/800) in Iowa, 3.2% (24/750) in Kansas, 5.0% (15/300) in Missouri, and 7.3% (42/575) in Texas. These findings indicate that *Campylobacter* is commonly prevalent in the U.S. feedlot cattle, and that clone SA constitutes a substantial portion of the commensal cattle *C. jejuni* population.

027P

Modeling considerations in the analysis of associations between antimicrobial use and resistance in beef feedlot cattle

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A number of sophisticated modeling approaches are available to analyze data regarding potential associations between antimicrobial use (AMU) and resistance (AMR) in animal health settings. All have their advantages and it is not clear which is most appropriate or whether all approaches yield consistent results.

We used advanced regression modeling to investigate AMU-AMR associations in faecal non-type specific *Escherichia coli* (NTSEC) isolates recovered from 275 pens of feedlot cattle. Both random effects mixed models and generalized estimating equations with alternating logistic regression were used to account for hierarchical non-independence of model outcomes. Measures of AMU were treated as both continuous and categorical exposure variables. In total, ten modeling strategies were employed to investigate AMU associations with resistance to chloramphenicol, ampicillin, sulfisoxazole, tetracycline and streptomycin.

Goodness-of-fit statistics did not show consistent advantage for one model type, and results were generally not concordant between models.

However, three associations were significant in all models. Recent parenteral tetracycline use increased the odds of finding tetracycline-resistant NTSEC (odds ratios [OR] ranged from 1.1 to 3.2); recent parenteral sulfonamide use increased the odds of finding NTSEC resistant to sulfisoxazole (ORs ranged from 1.4 to 2.5); and recent parenteral macrolide use decreased the odds of recovering ampicillin-resistant NTSEC (ORs ranged from 0.03 to 0.2). Nineteen additional AMU-AMR associations were found to be statistically significant in at least one of the 10 modeling strategies. Differential quantification of AMU (i.e. categorical vs. continuous) yielded the greatest discrepancy in model results.

These findings suggest that modeling alternatives of equal validity can lead to dramatically different study conclusions, which in turn emphasizes the need for caution when interpreting the results of a single model. Ideally, studies analyzing associations between AMU and AMR should utilize multiple modeling alternatives and compare results for consistency.

028P

Molecular and statistical analysis of *Campylobacter* spp. carriage and antimicrobial resistance in mammalian wildlife and livestock species from Ontario farms (2010)

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**Purpose:** The objectives of this study were to assess risk factors for the carriage of *Campylobacter* and antimicrobial resistant *Campylobacter* among livestock and mammalian wildlife on farms in Ontario, and to determine if *Campylobacter* subtypes are exchanged between wildlife and livestock based on molecular subtyping results.

**Methods:** Using data collected from a cross-sectional study of 25 farms in 2010, we assessed associations between *Campylobacter* and antimicrobial resistant *Campylobacter* carriage and the following explanatory variables using mixed logistic regression models: animal species, farm type, type of sample (livestock or wildlife), and *Campylobacter* species; the model included a random intercept to account for the farm where samples were collected. Isolates were subtyped using a *Campylobacter*-specific 40 gene comparative fingerprinting assay.

**Results:** A total of 92 livestock samples and 49 raccoon samples were collected with 72% (66 livestock) and 41% (20 raccoons) testing *Campylobacter* positive, respectively. Livestock *Campylobacter* isolates were significantly more likely to exhibit antimicrobial resistance (AMR) to  $\geq 1$  antimicrobial tested compared to wildlife *Campylobacter* isolates. *Campylobacter jejuni* was significantly more likely to exhibit AMR to  $\geq 1$  antimicrobial tested compared to *C. coli*. However, the resistant *C. jejuni* isolates were only resistant to tetracycline while *C. coli* exhibited multi-drug resistance patterns,  $\geq 2$  antimicrobials tested. Fecal samples from livestock were significantly more likely to test positive for *Campylobacter* than wildlife samples. Relative to dairy cattle, swine were at significantly increased odds of testing positive for *Campylobacter*. The odds of shedding *Campylobacter jejuni* was significantly greater in beef cattle compared to both dairy cattle and raccoons. Fifty unique subtypes of *Campylobacter* were identified, but only one subtype was found in both wildlife and livestock.

**Conclusions:** We concluded that the sharing of *Campylobacter* species between livestock and wildlife was uncommon based on identical subtype similarity and AMR patterns.

## Epidemiology and Animal Health Economics Posters

029P

Population dynamics of multi-drug resistant *Salmonella* in feedlot cattle treated with ceftiofur or chlortetracycline

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Multidrug-resistant (MDR) *Salmonella* are classified as a serious threat according to a 2013 report from the Centers for Disease Control and Prevention. Cases of human foodborne salmonellosis have not declined in recent decades and prudent use of antibiotics in animal agriculture is appropriate for preventing dissemination of MDR-*Salmonella* to humans; however, defining prudent use in the realm of treatment, control and prevention use remains difficult. We used experimental studies to assess the effects of metaphylactic use of injectable ceftiofur (CCFA) and in-feed chlortetracycline (CTC) on the dynamics of antibiotic-resistant *Salmonella* in feedlot cattle. Sixteen pens (11 steers/pen) were subjected to 4 regimens; 1) all 11 steers in 8 pens were treated with CCFA on Day 0; 2) 4 of these pens followed with intermittent CTC from Day 4 through Day 19; 3) 1 out of 11 steers in the remaining 8 pens were treated with CCFA on Day 0; 4) 4 of these pens received CTC as above. Feces collected from individual steers on Days 0, 4, 14, and 26 were spiral-plated on Brilliant Green agar for quantification and subjected to *Salmonella* isolation. Phenotypic resistance of isolated strains was analyzed via the TREK Sensititre® system. The mean *Salmonella* prevalence in feces before treatments was approximately 60%. All of the *Salmonella* isolated on Day 0 were pan-susceptible to the panel of 15 antibiotics. Treatments with both CCFA and CTC initially reduced both the prevalence and the total amount of *Salmonella* in feces ( $P < 0.05$ ); however, the proportion of MDR-*Salmonella* increased on Day 14 in steers treated with both CCFA and CTC, or CTC alone, suggesting a shift in population that remained. *Salmonella* isolated from the 3<sup>rd</sup> regimen pens did not show significant phenotypic change throughout the study period; in addition, the quantity of *Salmonella* remained stable ( $P > 0.05$ ). Our study suggests that antibiotic use in feedlot cattle may be contributing to a changing population of *Salmonella* in cattle feeding environments. Since most therapeutic antibiotic use in fed cattle occurs early in the feeding period, longer term studies that follow cattle to slaughter are required to assess any risk to food safety and public health.

030P

Tracking association between wild life and avian influenza using GPS-CDMA based Telemetry System

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**Purpose:** This study was aiming at understanding the role of wild life in transmitting avian influenza (AI) virus using global positioning system (GPS) - code division multiple access (CDMA) based telemetry.

**Methods:** The investigated areas are habitat for migratory birds, peripheral areas and poultry farms that the HPAI has been occurred. This research included capturing 20 wildcats and 300 rodents during 2 years, and investigating whether wild animals were infected by AI virus. The movement course of wild animals nearby AI occurred location was investigated with location based service (LBS) positional information. Based on positional information of 24 feral cats, collected by LBS, the area of activity and home range were analyzed. Recently developed tracking tools, including GPS and Mobile Phone based telemetry system were used.

**Results:** A total of 13 areas in 9 regions on a national scale are selected. In this region, total 52 feral cats and 324 rodents were captured and analyzed the infection possibility. No individual was found to be infected with AI plaque. The feral cats showed a maximum distance of movement for 1,700 meters, and they moved within 500 meters on an average. The average of home range was 58,000 square meters, and it could be up to 350,000 square meters at the largest. Estimates of habitat ranges increased at the wide-open area such as farm land and the terrace land on the river, and varied by sex and body size of the feral cats.

**Conclusions:** Our findings allow inferring that wild animals can mechanically transmit AI virus to the poultry farms located at distance away within their habit range. This study suggests the importance of controlling wild animals, including rodents, at least 2 kilometers of radius from an HPAI outbreak farm.

031P

Analysis of the temporal features of highly pathogenic avian influenza (HPAI) H5N8 epidemic in South Korea, 2014

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**Purpose:** The objective of this study was to describe the temporal patterns of highly pathogenic avian influenza (HPAI) occurrence in South Korea, 2014 and to identify potential factors for the introduction and spread of infection within the poultry population.

**Methods:** Animal & plant Quarantine agency (QIA)'s disease reporting data were used for this study, and the observational period was from 16 January to 25 July 2014. A farm was considered to be one case of HPAI if infection was confirmed by the detection of the HPAI virus using RT-PCR. Confirmed cases were analyzed by the daily, weekly, and monthly distribution of HPAI cases during the period and the epidemic curve. Survival plots of the susceptible poultry population and infection period of the cases were estimated based on HPAI H5N8 outbreak features.

**Results:** The results indicate that during the investigated period a sequence of two epidemic waves occurred in weekly analysis as distinct spatial and temporal clusters but the cases were reported constantly during first 2-months. According to estimated infection date, it is speculated that at least 50 farms were already infected before first reporting of HPAI. The patterns of epidemic curves based on case-reporting dates and estimated infectious dates were identical.

**Conclusions:** Information obtained from the temporal analysis associated farming areas show that the pathway of HPAI introduction into the farms and the subsequent pattern of spread in South Korea, 2014 fit the notion that wild birds migration and domestic poultry marketing contributed to HPAI virus introduction and diffusion.

## Epidemiology and Animal Health Economics Posters

032P

A needs-based assessment of the drivers of lost productivity for camels of eastern Ethiopia.

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Because of their relative drought resistance and high milk output, camels are an increasingly important livestock commodity of the pastoralist and mid-altitude farmers of East Africa. Unfortunately, the development of camel specific diagnostic services has not mirrored the growth of this livestock sector, leaving the industry unable to fully capitalize on the economic potential of its camel herds.

The immediate goal of this study was to understand the current limitations of camel productivity and disease in the Ethiopian context, with a subsequent goal of improving diagnostic medicine capacity for camels in Ethiopia. By first conducting a needs-assessment, we believe that capacity building can be conducted in a sustainable, beneficial, and socially acceptable fashion.

Camel herders and Community Animal Health Workers in the Afar and Somali Regional States of Ethiopia were interviewed using a participatory epidemiology approach, focusing on perception of camel productivity, limitations to productivity, mortality and morbidity information, seasonality of diseases, and reproductive success of camels. The possible social, cultural and geographic barriers to utilizing veterinary diagnostic testing were discussed. In addition, targeted sample collection and basic diagnostic testing of camels was conducted. Interviewees ranked the significance and seasonality of diseases, effect of diseases on production, and access to veterinary services. Field-testing included a physical exam, body condition score, fecal exam, FAMACHA score, packed cell volume, and a number additional tests based on clinical signs.

Camels were considered the most valuable form of livestock, with milk production being the camels' most important function. Significant diseases included orf/camelpox, respiratory disease, and trypanosomiasis. Veterinary services were extremely limited in these regions. Lack of veterinary diagnostic services in these regions has led to inadequate and/or inappropriate treatment. Increasing diagnostic capacity, focused on those diseases that most severely affect production, has the potential to significantly improve camel production in Ethiopia.

033aP

Examining the cross-species infectivity of human and swine specific torque teno viruses (TTVs)

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>Torque teno viruses (TTVs) are small, ubiquitous, circular single stranded DNA viruses that were discovered in post-transfusion non A-G hepatitis patients. Several species-specific torque teno viruses [TTVs] exist at high prevalence rates. In swine, TTVs are epidemiologically associated with other pathogens such as porcine circovirus strain 2 and porcine reproductive and respiratory disease syndrome virus. However, the role of TTV as a potential pathogen or in modulating host immune responses remains elusive. Similarly, it is not clear if cross-species infections of TTVs occur. To investigate whether swine and human TTVs can be detected in other species, a panel of 79 sera from humans and various domestic animals was screened using three different PCR detection systems; namely a swine specific, human specific, and universal TTV detection system. Surprisingly, swine-specific TTV DNA was detected in 100%, 40%, 80%, 50%, and 89% of human, sheep, dog, horse, and cattle samples respectively. Similarly, human specific TTV DNA was detected at 20%, 100%, 100%, 60% and 11% prevalence in swine, sheep, dog, horse, and cattle samples respectively. This preliminary data suggests that the DNA of TTVs circulates freely between species. Further studies are required to elucidate whether the presence of DNA correlates with productive cross-species infections.

## Immunology Posters

033P

Swine toolkit progress for the US Veterinary Immune Reagent Network.

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The US Veterinary Immune Reagent Network (US VIRN) was established to address the lack of immunological reagents specific for veterinary species. Efforts were targeted at swine, ruminants, poultry, equine and aquaculture species. The US VIRN grants have ended; data is still stored on the website [www.vetimm.org](http://www.vetimm.org). Our goal for porcine reagents was to produce bioactive cytokines and chemokines, and monoclonal antibodies (mAb) that function in ELISA, multiplex bead assays, and flow cytometric applications. Numerous swine chemokines and cytokines were cloned, expressed in *Pichia*, purified; most were shown to be bioactive using chemotaxis, upregulation of marker expression and/or cell stimulation assays. Recent products include IL-17A, IL-17F, IL-21, GM-CSF and sCD40. These products are available to collaborators and have been made commercially available through Kingfisher Biotech, Inc. [www.kingfisherbiotech.com/](http://www.kingfisherbiotech.com/). We are currently working on characterization of sets of mAbs reactive with interferon-beta (IFN $\beta$ ) and interleukin-13 (IL-13) produced at Univ. Massachusetts, Univ. Connecticut and Cornell Univ. Our goal is to develop new ELISA and fluorescent microsphere, Luminex bead, immunoassays for these proteins, the latter to be included in our current 7-plex swine cytokine assay. At Cornell Univ. a fusion protein expression system was used to generate material for immunizations for cell surface antigens, IFNAR and NK cell marker NKp44 (NCR2). New mAb for these targets have been produced and characterized. Efforts are still underway for developing an anti-poCD19. Since many swine cytokine and CD reagents are available commercially the website also includes a listing of those reagents and their sources. This project was funded by USDA NIFA proposal #2010-65121-20649, USDA NIFA/DHS #2010-39559-21860 grants and USDA ARS funds.



## Immunology Posters

034P

Induction of type-I interferons by a synthetic porcine reproductive and respiratory syndrome virus strain

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Type-I interferons (IFNs), mainly represented by IFN- $\alpha/\beta$ , play a critical role in innate resistance to viral infection. IFN- $\alpha/\beta$  also regulate the host adaptive immune response by promoting the differentiation of both CD4+ and CD8+ T cells, the induction of IFN- $\gamma$  as well as by suppressing TReg cells functions. Porcine reproductive and respiratory syndrome virus (PRRSV) is well characterized for its capacity to suppress IFN- $\alpha/\beta$  production. However, it has been recently reported that some naturally occurring PRRSV strains can stimulate, rather than suppress, type-I IFN response in cell culture although this does not seem to be a common phenotype. We report here the characterization of a synthetic PRRSV strain (designated as PRRSV-CON) that induces robust type-I IFN response in vitro. The PRRSV-CON genome was de novo synthesized based on the consensus sequence generated from 60 wild-type PRRSV full-genome sequences. High levels of IFN- $\alpha/\beta$  mRNA and interferon-stimulated gene 56 (ISG-56) protein were readily detected in cells that were infected with PRRSV-CON, but not in the cells that were mock-infected or those that were infected with our prototype PRRSV strain FL12. We have used reverse genetics to create different chimeric viruses by exchange different gene fragments of the PRRSV strain FL12 (e.i. suppressing type-I IFNs) with the corresponding genes of PRRSV-CON (e.i. inducing type-I IFNs). We are in the process of characterizing these chimeric viruses in order to identify which genes of the PRRSV-CON are responsible for type-I IFNs-inducing phenotype. Collectively, the results obtained from this study may provide important information for the rational development of the more effective PRRS vaccine.

035P

Failure of CTB fused to Porcine arterivirus M and GP5 proteins to enhance the porcine reproductive and respiratory syndrome virus GP5-specific antibody response in pigs

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The causative agent of Porcine reproductive and respiratory syndrome (PRRS), the PRRS virus (PRRSV), belongs to the Arteriviridae family. As the current commercial vaccines are incompletely effective to ensure protection against PRRS, a vaccine strategy using replicating but non-disseminating adenovectors (rAdVs) expressing the PRRSV M matrix protein in fusion with the neutralizing major epitope-carrying GP5 envelope protein was developed by us (Veterinary Research 44:17, 2013). Although this strategy enabled the production of GP5-specific antibodies (Abs), no PRRSV-specific neutralizing Abs (NAbs) were induced with rAdVs expressing either M-GP5 or M-GP5m (which is a mutant form of GP5 that was designed to theoretically enhance the production of PRRSV-specific NAbs). In this study we wished to determine whether the fusion of the cholerae toxin B subunit (CTB, known for its adjuvant effect) to the C-terminus of M-GP5m was able to enhance the Ab response to GP5 and to induce PRRSV-specific NAbs. Three rAdVs were generated and designated rAdV-GFP (expressing the green fluorescent protein and used as a negative control), rAdV-M-GP5m, or rAdV-M-GP5m-CTB. Three weeks-old pigs (five pigs per group) were immunized twice both intramuscularly (IM) and intranasally at 3-weeks intervals with the various rAdVs. Four additional pigs were inoculated IM at days 0 and 21 with 2 ml of the commercial Ingelvac PRRSV MLV vaccine inactivated 1 h at 56°C prior to the inoculation (designated hereafter inactivated vaccine). Serum GP5-specific Abs and PRRSV-specific NAbs were determined prior to immunization, and at subsequent post-primary immunization (PPI) days by ELISA and by a virus neutralization assay, respectively. Immunization of pigs with rAdV-M-GP5m or the inactivated vaccine generated similar GP5-specific Ab response from day 28 PPI. Unexpectedly CTB fused to the virus proteins had a severe negative impact on GP5-specific Ab production when compared to the use of M-GP5m alone. Moreover PRRSV-specific NAbs could not be detected in any pigs of all groups. Experiments are under way to delineate the mechanism associated to the negative effect of CTB.

036P

Advances in vaccine design: Developing a cross-conserved influenza vaccine for swine

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**Purpose:** Computer-driven algorithms enable accelerated vaccine design for emerging pathogens. iVAX is an integrated set of epitope-driven vaccine (EDV) design tools that is based on the EpiMatrix algorithm and has been extensively validated for human vaccine design. Comparable tools for the development of vaccines for food animals are not available. We used the pocket profile method to create T cell epitope-prediction matrices (PigMatrix) for common class I and class II Swine Leukocyte Antigens (SLA) alleles. The new matrices were integrated into iVAX to generate a comprehensive suite of vaccine design tools that can be used to produce EDV for pathogens affecting swine.

**Methods:** We applied PigMatrix to produce an influenza vaccine composed of conserved and immunogenic T cell epitopes. From seven circulating influenza A viruses (IAV), 28 cross-conserved class I and 20 class II epitopes were identified. Multi-epitope DNA vaccines encoding strings of class I and II epitopes separately were produced and pooled for pig immunizations. Animals were primed by intramuscular injection and boosted twice at three-week intervals. Pigs were challenged intranasally three weeks after the final boost with A/California/04/2009 (H1N1). **Results:** Peptides induced epitope-specific recall responses as evidenced by IFN- $\gamma$  production in PBMC cultures stimulated with individual or pooled peptides, demonstrating their antigenicity and validating the SLA matrices. Epitope-specific responses in DNA-vaccinated pigs were equivalent in magnitude to whole inactivated virus-induced responses in pigs immunized with quadrivalent inactivated vaccine (FluSureXP). In terms of lung lesions, temperatures, and viral loads, neither the DNA vaccine nor the commercial vaccine was protective against intranasal challenge.

**Conclusions:** These results provide proof-of-concept that PigMatrix may be applied to produce immunogenic vaccines for other important swine pathogen targets, including PEDv, PRRSV and ASF.

## Immunology Posters

037P

Performance assessment of a real-time polymerase chain reaction assay for porcine epidemic diarrhea virus to assess PEDV transmission in growing pigs

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PEDV was first diagnosed in the U.S. in April 2013 as sporadic cases of diarrhea in young piglets with high mortality. Real-time RT-PCR is a high throughput test system that has potential to detect PEDV during the acute phase of the infection or pre-seroconversion. A study in nursery pigs was conducted to assess the transmission potential of young pigs experimentally-infected or experimentally-exposed to PEDV. On day 0 (D0), a 4-week-old pig was challenged with PEDV and 14 naïve contacts were comingled. On D7, 9 contact pigs were moved to a new room to serve as the principal virus reservoir group (PG), and comingled with 1 naïve age-matched sentinel (S1). Three days later, the S1 pig was moved to a separate room until necropsy. This process was repeated on D14, 21 and 28 with pigs S2, S3 and S4. On day 49, 5 naïve age-matched pigs (N) and the PG were challenged (N/C, PG/C) with homologous virus and euthanized on D78. A daily rectal swab was collected from each pig and tested for PEDV using real-time RT-PCR to detect the N gene (gN) and the S gene (gS) using commercial chemistry. Detection limits and threshold cycle (Ct) values of real-time RT-PCR were assayed for PEDV samples and positive controls for both gN and gS. The coefficient of variation determined based on the replicates (intra-assay variation) ranged from 0.00% to 2.65% and inter-assay variation had an average of 2.75%. Real-time RT-PCR PEDV assay results are visualized in a heat map where positive is  $Ct \leq 34.99$ . All PG pigs were real-time RT-PCR-positive from D3-11, with some intermittently positive to D42. Following challenge at D49, all PG pigs were negative PC and all N/C pigs were positive by D52 days. 3/5 pigs until 13 days PC. PEDV RNA was detected in S1 and S2 within 1 day of contact, but not detected in S3 or S4. Real-time RT-PCR positive rectal swabs collected after D21 were tested for infectious virus using bioassay techniques. A critical need for the current PEDV surveillance program is the rapid detection of PEDV. The present study evaluated the real-time RT-PCR assay to detect PEDV infection in the transmission potential of young pigs experimentally-exposed to PEDV.

038P

Use of oral fluids for the detection of IgG, IgA, and IgM antibodies against porcine epidemic diarrhea virus (PEDV) from experimentally infected weaned pigs

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Porcine epidemic diarrhea virus has caused significant economic loss to the US swine industry since it emerged in 2013. As swine oral fluids have been frequently used for virus and antibody detection, this study investigated the use of oral fluids as a valid sample matrix for the detection of antibodies against PEDV in a herd of experimentally infected pigs. Ninety-six 3-week-old weaned pigs without prior PEDV exposure were randomly allocated to one of 2 control groups (n=40) and 4 challenged groups (n=56). Challenged pigs were orogastrically inoculated with 103 PFU of PEDV isolate (US/Iowa/18984/2013). Control pigs received volume-matched virus-free cell culture media. Oral fluids were collected from all groups daily for the first week post-inoculation and then twice a week through 56 days post-inoculation (dpi) using a rope collection method. Sera were collected from individual pigs on dpi 0 and weekly. Both oral fluids and sera at a dilution of 1:2 and 1:50, respectively, were tested for IgM, IgG, and IgA by an indirect ELISA using whole virus antigens of the challenge strain. ELISA was performed using a peroxidase-TMB substrate system. Colorimetric reactions were measured as OD values at 450nm. Statistical analyses were performed on normalized OD values to compare control versus challenged group averages as well as oral fluids versus sera. Both control and challenged pigs showed no signs of diarrhea prior to inoculation. While control pigs continued to be asymptomatic for the duration of the study, PEDV-challenged pigs displayed watery feces starting at 2 dpi, with most pigs recovering by 10 dpi while still shedding the virus in feces for longer. For both sera and oral fluids, average IgG and IgA OD values were statistically greater for the challenged groups beginning at 10 dpi and remained significantly greater through the duration of the study, while IgM values remained low. Serum IgA and IgG values followed the same overall trend of increasing to peak levels at 21-28 dpi followed by a steady decline, while oral fluid IgA values remained high. This study demonstrated that oral fluids are a valid sample type for detection of IgG and IgA antibodies against PEDV using a whole-virus based indirect ELISA.

039P

Inhibition of Type I Interferon Induction by Nonstructural Proteins of Porcine Epidemic Diarrhea Virus.

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Type I interferons (IFN- $\alpha/\beta$ ) play a key role for the host antiviral state. Some viruses in the families *Arteriviridae* and *Coronaviridae* in the order *Nidovirales* have been shown to down-regulate the production of type I IFNs during infection. Porcine epidemic diarrhea virus (PEDV) has emerged in the United States since 2013 and has spread rapidly to most pig-producing States posing significant economic concerns. To study the ability of PEDV for innate immune modulation and to identify the viral components responsible for this activity, all 16 nonstructural protein (nsp) genes of PEDV were cloned and screened for IFN- $\beta$  responses. Nsp1 through nsp16 genes were individually amplified from the viral genome and inserted into pXJ41 eukaryotic expression vector using FLAG as a tag for detection. Their gene expression in cells was confirmed by western blot analysis and immunofluorescence (IFA), and the modulation of IFN production was determined by luciferase reporter assay and vesicular stomatitis virus (VSV)-GFP bioassay. Of 16 nsps, nsp1, nsp7, nsp14, and nsp15 were evident to inhibit IFN, interferon regulatory factor 3 (IRF3), and NF- $\kappa$ B promoter activities. The inhibitions were not due to the inhibition of IRF3 nuclear translocation, suggesting a nuclear event for IFN modulation. Of note, nsp1 significantly impaired the activation of IFN- $\beta$  promoter when stimulated by IPS-1, TRAF3, and IRF3. Furthermore, nsp1 degraded the CREB-binding protein (CBP) in the nucleus, inhibiting the formation of enhanceosome thus resulting in the suppression of IFN production. In conclusion, our data show that nsp1, nsp7, nsp14, and nsp15 are the PEDV IFN antagonists, and at least one mechanism for IFN inhibition by PEDV is CBP degradation by nsp1.

040P

Safety and antibody response of pigs to an experimental Porcine Epidemic Diarrhea Virus (PEDV) Vaccine, Killed Virus

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## Immunology Posters

(040P continued)

A study was conducted to evaluate the safety and antibody response of an experimental killed PEDV vaccine in non-pregnant sows. PEDV negative sows were vaccinated twice, 3 weeks apart with the experimental vaccine (n=23) or an adjuvant placebo (n=3). Sera, collected on Days -1, 21, 30 and 35, were tested for PEDV antibodies by 4 methods. An indirect ELISA to detect antibodies to purified recombinant S1 protein, a Beta serum neutralizing (SN) assay and an indirect fluorescent antibody (IFA) assay were conducted at Zoetis. Sera were also sent to the Animal Disease Research and Diagnostic Laboratory at South Dakota State University, Brookings, SD to test for fluorescent focal neutralization (FFN) titers. Pearson correlations between the assays were calculated. *In vivo* procedures occurred according to state, national, or international regulations after ethical review by Zoetis's IACUC. No untoward local or systemic reactions attributable to the vaccine were observed in the vaccinated sows. Antibody titers of the placebo controls remained at pre-vaccination levels at all post-vaccination time points and vaccinated sows had detectable antibodies to PEDV at all post-vaccination time points as measured by the 4 assays. There were correlations between the assays (Table 1). Under the conditions of this study, the experimental vaccine was both safe and immunogenic, inducing neutralizing antibodies (SN and FFN), antibodies to the whole virus (IFA) and antibodies to the S1 protein.

Table 1. Correlation of PEDV antibody assays P>.

041P

Antigenic relationships among porcine epidemic diarrhea virus and transmissible gastroenteritis virus strains.

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Porcine epidemic diarrhea virus (PEDV) and transmissible gastroenteritis virus (TGEV) are economically important swine enteropathogenic viruses. They belong to the *Alphacoronavirus* genus within *Coronaviridae*. These two viruses induce similar clinical signs and pathological lesions in newborn piglets, but they are presumed to be antigenically distinct. However, serological cross-reactivity among different strains of PEDV and TGEV has not been examined comprehensively. In the present study, two-way antigenic cross reactivity studies between the prototype PEDV (CV777) strain, 3 distinct US strains of PEDV (PC22A, Iowa106 and PC177) and 2 representative TGEV strains (Miller and Purdue) were conducted by cell culture immunofluorescent (CCIF) assay. One-way cross reactions were observed between TGEV Miller hyperimmune pig antisera and all PEDV strains. ELISA using monoclonal antibodies and prokaryotic recombinant PEDV/TGEV N protein, Western blot and sequence alignment showed at least one epitope on the N-terminal region of PEDV/TGEV nucleoprotein (N) contributed to this cross-reactivity. None of the pig TGEV antisera neutralized PEDV and *vice versa*. In addition, comparison of PEDV CV777 and other PEDV US strains revealed antigenic and biologic diversity consistent with the genetic differences reported. PEDV CV777 induced greater cell fusion in Vero cells than the US PEDV strains. All 3 US PEDV strains had comparable IgG and viral neutralizing (VN) antibody titers with the same panel of antibodies. However, 2 pig PEDV CV777 and 2 pig PEDV PC22A convalescent antisera showed 4- to 16-fold higher homologous antibody titers than heterologous antibody titers in CCIF and VN assays. Furthermore, all trypsin-treated PEDV strains showed similar hemagglutination (HA) activity against rabbit red blood cells. Our results provide direct evidence supporting N protein based antigenic cross-reactivity between PEDV and TGEV that could compromise some diagnostic assays. Information on the biologic and antigenic diversity among PEDV strains is helpful for the development of diagnostic immunoassays and effective future vaccines.

042P

Expression of Mycoplasma hyopneumoniae P97c recombinant protein in Escherichia coli and purification for putative adjuvant activity analysis  
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Adjuvants are compounds that increase and/or modulate the immunogenicity of an antigen and induce strong and lasting immune responses. P97 protein of Mycoplasma hyopneumoniae (M. hyopneumoniae) was characterized as an adhesion protein of this organism to ciliated cells of the respiratory tract in pigs. Our previous studies indicated that the C-terminus of P97 (P97c) tagged to other proteins [such as GP5 of the porcine reproductive and respiratory syndrome virus or the capsid (Cap) protein of the Porcine Circovirus type 2], and delivered through adenovectors enhances the antibody response in mice, suggesting an adjuvant activity of P97c (Vaccine 31:2698-2704, 2013). However the availability of purified recombinant P97c for a simpler application as an immune adjuvant and to delineate its specific mechanisms is required. In this study, the sequence encoding the P97c was cloned into the pET21b expression vector and the protein was expressed in Rosetta Gami bacteria. Protein expression was low and several cleaved forms of P97c were found. Therefore the P97c cDNA sequence was cloned into the pET22b vector harboring a periplasmic export sequence. P97c was expressed in BL21 bacteria at high levels and then purified by using size exclusion chromatography. Experiments are underway to determine the biological activity of the purified P97c protein.

043P

Polyfunctional cytokine responses by central memory CD4+ T cells in response to bovine tuberculosis.

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CD4 T cells are crucial in immunity to tuberculosis (TB). Polyfunctional CD4 T cells simultaneously produce interferon-gamma (IFN- $\gamma$ ), interleukin-2 (IL-2) and tumor necrosis factor-alpha (TNF- $\alpha$ ) and play relevant roles in several chronic infections, including human TB and HIV. Mycobacterium bovis infection of cattle elicits ex vivo polyfunctional T cell responses. Long-term (i.e., 14 days) cultured IFN- $\gamma$  responses by peripheral blood mononuclear cells are used as a correlate of T cell central memory (Tcm) responses in both humans and cattle. The objective of the present study was to assess polyfunctional T cell responses by Tcm in cattle (n = 6) in response to aerosol M. bovis infection. For long-term culture, PBMCs collected from infected cattle were stimulated with M. bovis purified protein derivative (PPDb), rESAT-6:CFP-10 (E:C) and peptide cocktails of Tb10.4 and Ag85A for 13 days with periodic addition of fresh media and rIL-2. After stimulation, the expression of CD4, CD45RO, CCR7, IL-2, IFN- $\gamma$ , TNF- $\alpha$  and cell viability were analyzed by flow cytometry. Long-term T cells responding to E:C consisted of 11% effectors (CD4+ CD45RO- CCR7-), 22% effector memory (CD4+ CD45RO+ CCR7-), and 76% central memory (CD4+ CD45RO+ CCR7+) phenotypes. In regard to the cytokine profile, 53% of cells producing cytokines expressed both IFN- $\gamma$  and TNF- $\alpha$ , 42% expressed all three

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cytokines, 13% expressed both TNF- $\alpha$  and IL-2, and 7% expressed IL-2 and IFN- $\gamma$ . Cells producing only IFN- $\gamma$ , IL-2, or TNF- $\alpha$  represented, 9%, 5% and 1%, respectively. These findings demonstrate that 14-day cultured cells (primarily Tcm cells) exhibit polyfunctional responses consisting mainly of cells co-producing IFN- $\gamma$  and TNF- $\alpha$  or IL-2, IFN- $\gamma$  and TNF- $\alpha$ .

044P

Adenosine triphosphate interactions with bovine purinoceptor 7

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Extracellular adenosine triphosphate (ATP) is involved with mediating inflammation in many tissues throughout the body. Of the purinergic receptors that interact with ATP, P2X purinoceptor 7 (P2X<sub>7</sub>) is the one most associated with the classic tissue changes associated with inflammation, especially permeability changes. In our present research, we have tried to characterize this interaction using bovine epithelial cells and different inhibitors of the P2X<sub>7</sub> receptor. The bovine mammary gland epithelial cell line, Mac-T, was used as the epithelial cell model. Mac-T cells were incubated with combinations of ATP and the P2X<sub>7</sub> inhibitor, and permeability changes in the monolayer were measured using a transepithelial electrical resistance system. In addition, the ability of ATP to activate the P2X<sub>7</sub> receptor was evaluated by measuring the movement of Ca<sup>2+</sup> and the Yo-pro dye marker into the cell using a fluorescent plate reader. ATP exposure did induce a marked increase in epithelial monolayer permeability, which was reversible by the addition of P2X<sub>7</sub> receptor antagonists. ATP exposure did not increase movement of Ca<sup>2+</sup> from the extracellular space into the intracellular space of the epithelial cell. In contrast, ATP exposure did induce a modest influx of Yo-pro into the interior of the cell. In conclusion, extracellular ATP does appear to influence permeability changes in bovine epithelial cells. This process potentially assists the movement of inflammatory cells and other blood products across epithelial cell layers. The P2X<sub>7</sub> receptor is a ligand-gated ion-channel. The lack of calcium movement into the cell after ligation was unexpected and is currently being investigated.

045P

Quantification of oxylipid profiles in bovine mammary tissue and milk during *Streptococcus uberis* mastitis

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In response to bacterial infection in the mammary gland, the onset and resolution of inflammation must be tightly regulated to prevent tissue damage. *Streptococcus uberis* is a major pathogen of concern as it results in severe tissue damage and significant milk production losses due to an uncontrolled and ineffective inflammatory response. A complex repertoire of oxidized fatty acids, or oxylipids, can orchestrate the inflammatory response. Previous coliform mastitis trials demonstrate an abundance of a limited number of pro-inflammatory oxylipids, however the complete profile of oxylipids during *S. uberis* mastitis is unknown. Thus, the hypothesis for the current study is during *S. uberis* mastitis, pro-inflammatory oxylipids will increase resulting in an imbalance of pro- and anti-inflammatory oxylipids. A targeted array of oxylipids was used to quantify profiles at the onset of clinical mastitis and temporally during *S. uberis* mastitis initiation and progression. At the onset of clinical symptoms, there was an increase in 6-keto PGF<sub>1 $\alpha$</sub> , PGE<sub>2</sub>, PGF<sub>2 $\alpha$</sub> , and 5-oxoETE in *S. uberis*-infected mammary tissue. Interestingly linoleic acid metabolites predominated concomitant with increased 9- and 13-HODE and increased ratio of 9-HODE:9-oxoODE. There was no change in the temporal expression of oxylipids in milk samples from infected cows. Cows that were challenged with *S. uberis* but did not establish infection, however, demonstrated an increased ratio of 13-HODE:13-oxoODE and 13-HODE:9-HODE in milk, whereas cows that did establish an infection demonstrated increased total HODE synthesis. *In vitro* exposure of bovine mammary endothelial cells to 13-HODE did not significantly change inflammatory phenotype. In both tissue and milk profiles, the ratio of linoleic acid-derived metabolites was different suggesting the balance of oxylipids may influence disease pathogenesis and highlights the need for continued focus on regulation of inflammation by oxylipids.

046P

Role of strategic vaccination of dairy cows during the non-lactating period on enhancing serum and milk antibody titers during early lactation

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In dairy herds that have controlled contagious mastitis pathogens, *Streptococcus uberis* accounts for a significant proportion of mastitis in lactating and non-lactating cows. An important factor regarding immunological protection against bovine mastitis is how to induce protective immunity when dairy cows are highly susceptible to mastitis such as during the non-lactating and periparturient periods. The aim of this study was to determine intramammary immune responses during the periparturient and early lactation periods via strategic vaccinations conducted 28 days before drying off (D-28), at drying off (D-0) and 21 days before calving (C-21) with *Streptococcus uberis* antigen. A total of 40 dairy cows in the 1st or 2nd lactation were divided into three groups of 20 cows. Cows in group 1 were vaccinated with *S. uberis* antigen and cows in group 2 were injected with PBS plus adjuvant and used as a control. Cows were vaccinated at D-28, D0 and C - 21 days relative to drying off. Blood and milk samples were collected before and at each vaccination as well as at calving (C-0), 7 days after calving (C+7), and 14 days after calving (C+14) and analyzed for antibody titers. Results showed that serum and milk total IgG titers increased after each vaccination with highest titers achieved at calving (C0) in cows vaccinated with *S. uberis* antigen as compared to control cows. Similarly, serum and milk IgG1 and IgG2 titers were increased after each vaccination in cows vaccinated with protein extracts reaching its highest level at calving compared to control cows. However, both serum and milk titers start decreasing 7 days after calving which indicates the need for re-vaccination of cows at certain time interval to prevent such a drop in serum and milk titers. Thus, strategic vaccinations of dairy cows 28 days before drying off, at drying off and 21 days before calving induced increased antibody titers in serum and milk which reach its highest level at calving. Therefore, maintaining this high titer by re-vaccinating cows strategically when their titer start decreasing may prevent occurrence of *S. uberis* mastitis throughout the lactation cycle.

047P

Novel adjuvant enhances immune response to *Leptospira* bacterin

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Leptospirosis infection of cattle is one of the major causes of reproductive failure, resulting in abortions, poor fertility, weak calves, reduced growth and milk production. Cattle are reservoir hosts, can develop long term chronic infections that transmit *Leptospira* organisms to other

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cattle, other animals and humans who come in contact with them. Vaccines against *Leptospira borgpetersenii* serovar Hardjo, the most common serovar found in cattle, protect against reproductive failure, but their efficacy for preventing colonization remains controversial. Recent studies have indicated that cell-mediated responses in addition to antibody are necessary for development of effective immune responses to serovar Hardjo. The immune response in 18 month old cattle to two commercial monovalent *Leptospira* vaccines was compared to a monovalent whole cell bacterin delivered in a novel water-oil-water adjuvant. The animals receiving the novel vaccine preparation had a significant humoral and cell mediated immunity as early as 4 weeks following the first immunization, before the second booster. Antibody titers persisted up to 32 weeks following immunization, and were higher than animals receiving either commercial vaccine product. These animals also exhibited cellular proliferative responses to antigens from multiple *Leptospira* strains. No vaccinated animals were found to be shedding *Leptospira* in the urine for up to 8 weeks post-challenge. The use of a novel water-oil-water adjuvant preparation enhanced immune responses to a whole-cell bacterin of *Leptospira borgpetersenii* serovar Hardjo, with animals developing titers and proliferative responses soon after the first dose, responses persisted for 32 weeks and were robust enough to recognize antigens across serovars. This adjuvant should be considered for future application in *Leptospira* vaccine development.

048P

Comparison of a novel point-of-care diagnostic, PCRun, with real time *Leptospira* PCR for detection of *Leptospira* antigen in canine samples

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Infection with *Leptospira* is a worldwide zoonotic problem, with reservoirs of infection including both domestic and wild animals. Transmission of the organism is due to contact with infected urine and contaminated environments. Many of the clinical signs associated with Leptospirosis are non-specific, emphasizing the need for accurate and appropriate diagnostic testing to enable proper treatment and the prevention of infection of susceptible animals and people. There are currently several different types of tests to detect *Leptospira*, each with limitations. The limitations include low sensitivity and/or specificity, dependence on amount of *Leptospira*, slow growth, test cross-reactivity, appropriate collection time of samples, trained staff to perform and interpret tests, test costs, availability, etc. The current study evaluated a new technology for early *Leptospira* antigen detection, the PCRun Canine Pathogenic *Leptospira* Molecular Detection Kit (PCRun). This test was compared to *Leptospira* real time PCR as performed by the Wisconsin Veterinary Diagnostic Laboratory (WVDL). This new technology (PCRun), provided by Biogal, was designed to be a point-of-care diagnostic, eliminating the need for complex equipment and highly trained staff and shortening result turnaround time while maintaining high levels of test sensitivity and specificity. The canine samples used in testing were prepared laboratory aliquots that were designed to mimic situations in the field (e.g. different sample types and concentrations, various anticoagulants, multiple *Leptospira* serovars, etc.). This study showed a high level of sensitivity and specificity and excellent correlations between the test methods for most blood and urine samples. The PCRun had the advantage of not being negatively affected by the presence of heparin as an anticoagulant. Neither the real time PCR nor the PCRun detected non-pathogenic *Leptospira*.

049P

Pre-clinical determination of therapeutic protein immunogenicity for companion animals

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Purpose: With the expansion of veterinary medicine to include monoclonal antibodies and protein therapeutics, so comes the risk of anti-therapeutic immune response. We propose that in silico immunogenicity screening technology can and should be applied to veterinary biologic candidates.

Methods: Using the EpiMatrix T cell epitope mapping system, we have developed an interactive in silico screening and optimization platform that evaluates the overall immunogenic potential of a biologic as well as identifies individual T cell epitope clusters contributing to its immunogenicity. In contrast to other immunogenicity prediction tools, our platform considers the contribution of regulatory T cell epitopes (Tregitopes) to immunogenic potential. Tregitopes are highly conserved T cell epitopes derived from IgG that we and others have shown activate regulatory T cells and promote tolerance induction to associated antigens.

Results: We have demonstrated the correlation of available clinical immunogenicity data with Tregitope-adjusted immunogenicity scoring for twenty approved MAbs. We have developed experimental predictive models for murine, porcine, and feline T cell epitopes, and both human and species-specific Tregitopes have been shown to stimulate tolerogenic response in mice and non-human primates.

Conclusions: Incorporating companion animal T cell epitope mapping into our established platform for immunogenicity prediction for therapeutic protein products would represent a significant advance in safety screening for veterinary drug candidates.

050P

Highly accurate prediction of immunodominant B-cell epitopes of *Chlamydia* species using physicochemical and evolutionary properties of proteins

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Prediction of B cell epitopes by B-cell epitope prediction algorithms is

inaccurate. Using mono-specific mouse hyperimmune sera against all 9 chlamydial species, we developed biotinylated peptide immune assays for detection of species-specific anti-chlamydial antibodies. Compared to assays using whole or recombinant chlamydial antigens, these peptide ELISAs provide equal sensitivity, but without cross-reactivity. Using data from the large number of peptides tested, we developed bioinformatics approaches to predict immunodominant B-cell epitopes with high accuracy. We selected the 18 most immunodominant proteins that showed reactivity with antisera, and which contained at least one peptide sequence with high antibody reactivity as well as one peptide with no antibody reactivity. For ranking of 77 algorithms, predicted scores of 21 amino acid (AA) sliding windows of the annotated proteins were used in discriminant analysis. Functional B cell epitopes were 1.27- to 1.87-fold enriched for amino acids E, D, T, A, P, and 0.20- to 0.79-fold depleted of K, H, M, L, Y, W ( $p < 0.001$ ). B-cell epitopes had 87% increased intrinsic disorder tendency, i.e. undefined 3-dimensional structure in the

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natively folded protein, than non-epitope regions ( $p < 10^{-5}$ ). Backbone torsion angle fluctuations of the epitopes were 78% higher, and AA mobility was 77% higher ( $p < 10^{-5}$ ). The second best parameter was the mutation rate expressed as relative sequence polymorphism which was 80% higher in the epitope regions ( $p < 10^{-5}$ ). Increased relative solvent accessibility (surface exposed tendency) was the third best parameter to predict B-cell epitopes. Epitopes were also enriched for predicted coils/loops (64%) and had high hydrophilicity scores (57%). Disorder tendency combined with sequence polymorphism achieved B cell epitope prediction with 80% sensitivity and 80% specificity. Sixteen AA-long peptides produced a 20-fold higher signal than 8-11 AA-long peptides, and 20-40 AA-long peptides produced a 3-fold increased signal over 16 AA peptides ( $p \leq 0.01$ ). These findings will help to identify optimal peptide antigens and B cell epitopes in *Chlamydia* spp., and in serology of infectious agents in general.

051P

1 $\alpha$ ,25-Dihydroxyvitamin D3 inhibits differentiation and bone resorption of osteoclasts derived from Wistar rat bone marrow mononuclear cells  
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The steroid hormone 1 $\alpha$ ,25-dihydroxyvitamin D3 [1 $\alpha$ ,25-(OH)2D3] plays an important role in maintaining a balance in calcium and bone metabolism. This investigation aims to study the effects of 1 $\alpha$ ,25-(OH)2D3 on osteoclast formation and bone resorption.

Osteoclast differentiation was induced in bone marrow-derived mononuclear cells from Wistar rats with the addition of macrophage colony stimulating factor and receptor activator for nuclear factor- $\kappa$ B ligand in vitro. Cells were then treated with 1 $\alpha$ ,25-(OH)2D3 at 10-7mol/L, 10-8mol/L, or 10-9mol/L. Osteoclasts were identified using tartrate resistant acid phosphatase staining and activity monitored in the absorption lacuna by scanning electron microscope. Expression levels of functional proteins related to bone absorption including CAII, CK, and MMP-9 were evaluated by Western blot.

The results showed that 1 $\alpha$ ,25-(OH)2D3 inhibited the formation and activation of osteoclasts in a dose-dependent manner and downregulated the expression levels of bone absorption-related proteins. The number of OCs with 1 $\alpha$ ,25-(OH)2D3 at 10-7mol/L, 10-8mol/L, or 10-9mol/L was  $5.3 \pm 0.58$  cells/field,  $2.7 \pm 1.15$  cells/field and  $0.7 \pm 0.56$  cells/field, respectively (\* $P < 0.05$  or \*\* $P < 0.01$ ). After 1 $\alpha$ ,25-(OH)2D3 treatment, distribution of F-actin changed and decreased. The area of the lacuna in the groups treated with 1 $\alpha$ ,25-(OH)2D3 was smaller than those in the control group. In contrast, the expression levels of CAII, Cathepsin K and MMP-9 decreased in the groups treated with 1 $\alpha$ ,25-(OH)2D3 significantly or very significantly (\* $P < 0.05$  or \*\* $P < 0.01$ ).

The steroid hormone 1,25-Dihydroxyvitamin D3 [1,25(OH)2D3] stimulates bone resorption, in part, by regulating the number of osteoclasts, the principal resorbing cells of bone.

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052P

Comparative in vivo and in vitro studies of porcine rotavirus G9P[13] and human rotavirus Wa (G1P[8]) in gnotobiotic pigs.

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Rotavirus (RV) is a zoonotic pathogen that infects both children and young animals, including piglets and calves. Rotavirus is prevalent in pigs in the U.S. causing significant economic losses due to diarrhea in nursing and weaned piglets. Some commercially available vaccines lack efficacy and no anti-viral treatments are available. Recently we have shown that porcine group A rotavirus (PRV) G9P[13] strains are dominant in Ohio. Here we report results of *in vivo* and *in vitro* experiments to characterize a representative PRV G9P[13] strain. Virus shedding, clinical signs and homologous/heterologous protection were assessed *in vivo* using gnotobiotic (Gn) pigs. PRV G9P[13] induced diarrhea of similar severity to that of human RV (HRV) Wa G1P[8], but longer fecal virus shedding was still detectable by post-inoculation day (PID) 10. Unlike HRV Wa inoculated pigs challenged with PRV G9P[13], PRV G9P[13] inoculated Gn pigs had greater protection against homologous/heterologous RV diarrhea and shedding. Additionally, PRV G9P[13] induced longer viremia ( $\geq$  PID5) than HRV Wa. The sera (PID31) of Gn pigs inoculated with PRV G9P[13] contained high levels of homologous, but low levels of heterologous virus neutralizing (VN) antibodies against several human and porcine RVs. This suggests that other mechanisms facilitated the complete protection observed *in vivo* against the heterologous HRV Wa challenge. Using an immunofluorescence test we confirmed that PRV G9P[13], but not HRV Wa G1P[8], infected porcine peripheral blood monocyte-derived dendritic cells. This suggests that the pig origin PRV G9P[13] may infect a wider range of porcine cells, including immune cells, compared to HRV Wa. This may facilitate more efficient dissemination and persistence of PRV G9P[13] in swine. More studies are underway to clarify what types of immune cells are susceptible to PRV G9P[13] and to study their immune responses post-infection. Additionally, PRV G9P[13] strains will be further assessed in cross-challenge studies of other swine RVs to evaluate them as vaccine candidates.

053P

Colonization dynamics and effect of human rotavirus infection on defined commensal microflora in a gnotobiotic (Gn) pig model

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The richness and diversity of infant's microflora influences the resistance to infectious diseases postnatally. However, infant microflora succession when infected with human rotavirus (HRV) has not been investigated. Because of the intricacy involved, most studies have focused on investigating the defined commensal microflora (DMF) in less complex germ free animals. The objective of this study is to investigate the colonization dynamics of predominant infant bacterial community (DMF) comprising of *Enterococcus faecalis*, *Lactobacillus brevis*, *Streptococcus bovis*, *Escherichia coli* Nissle (EcN), *Bifidobacterium adolescentis*, *B. longum*, *Bacteroides thetaiotaomicron*, and *Clostridium clostridioforme* and the effect of virulent HRV (VirHRV) infection on the succession of these bacterial populations in a gnotobiotic (Gn) pig model. The Gn piglets were colonized with DMF (1X10<sup>5</sup>CFU each strain) at 5 days of age. A subset of pigs was euthanized on post challenge days (PCD) 5 and 21 to monitor the progression of DMF colonization. Before VirHRV challenge (PCD0), the duodenum was colonized with diverse bacterial spp. (5 of 8 spp.); however, EcN was the predominant colonizer in the jejunum, ileum and colon. At PCD5, pattern of bacterial diversity remained similar in the duodenum and jejunum irrespective of treatment; however, ileum and colon showed suppressed diversity of

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bacterial population in the DMF+VirHRV group. The VirHRV challenged pigs also showed increased *S. bovis* and *L. brevis* population, and decreased *EcN* population across the small intestine and colon compared to DMF group at PCD21. In conclusion, our study indicates that (i) in early stage (PCD5) of HRV pathogenesis there may be a suppression of bacterial diversity in ileum, that may likely predispose for enteric infection; (ii) in later stage (PCD21) although bacterial diversity was restored, the HRV infection affected the bacterial population structure.

054P

A fusion protein of *Escherichia coli* heat-labile toxoid (LT<sub>R192G</sub>) and spike protein epitopes of the porcine epidemic diarrhea virus induced neutralizing antibodies against PEDV

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**Purpose:** Porcine epidemic diarrhea virus (PEDV), a highly contagious porcine enteric pathogen, recently emerged in the US, and caused significant economic losses to the US swine producers. Currently, there is no effective prevention measure against PEDV in the US. Vaccination would be the most practical and effective approach to control PEDV. However, no vaccine has been developed in the US, and an effective vaccine is in great need.

**Methods:** In this study, we selected B-cell epitopes of the spike (S) protein of PEDV and constructed a fusion protein with heat-labile toxoid (LT<sub>R192G</sub>) of *Escherichia coli*, examined antigenicity of this fusion protein in mouse IP immunization, and evaluated potential application in vaccine development against PEDV.

**Results:** Data showed that immunized mice developed antibody responses to the PEDV S protein and also the LT toxin. Moreover, serum sample of the immunized mice showed neutralizing activity against the PEDV.

**Conclusions:** Results from this study suggest that this fusion antigen may be used in vaccine development against PEDV.

055P

Nucleocapsid protein of porcine epidemic diarrhea virus enhances viral replication *in vitro*

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The recent outbreak of porcine epidemic diarrhea virus (PEDV) has caused significant challenge to US swine industry. The isolation and propagation of PEDV in cell culture is the first step toward development of vaccines and diagnostic tests. However, the natural characteristics of the virus pose certain difficulties on the adaptation and passage of PEDV in cell cultures. In this study, we demonstrated that nucleocapsid (N) protein of PEDV has the ability to enhance infectivity of PEDV. In transfected cells expressing N protein, the PEDV grows to 10-fold higher peak viral titer, in comparison to the virus titer in untransfected cells. Further analysis showed that N protein interacts with nonstructural protein 3 (nsp3) and localized to the viral replication-transcription complex (RTC). The nsp3-N interaction was mapped to the N-terminal 160 amino acids region containing ubiquitin-like domain of nsp3 and the serine- and arginine-rich central region of N protein. These results support a previously established model of murine coronavirus, in which the N-nsp3 interaction serves to tether the viral genome to the newly translated RTC at early stage of infection, which could be a mechanism for N-protein dependent enhancement of PEDV infectivity.

056P

Pilot study of comparative immunology and cross-protection of a US spike-insertion deletion (S-INDEL) strain of PEDV and original US PEDV strain.

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Porcine epidemic diarrhea (PED) is an acute gastrointestinal disease caused by a coronavirus, PED virus (PEDV). PED causes severe morbidity and mortality in young pigs. There are limited experimental studies characterizing immune responses to US PEDV strains. We investigated immune responses and cross protection provided by a reportedly mild strain of PEDV (S-INDEL) against challenge with original PEDV. Two litters of three day old suckling pigs were inoculated orally with the S-INDEL PEDV (n=16), one litter with the original high virulent PEDV (n=11) and one litter with mock (n=6). The piglets were bled weekly to collect serum and serum and milk of sows were collected weekly. The surviving pigs were challenged with the virulent virus at 21 days post infection. Serum and milk antibodies were measured by sandwich ELISA using the original US PEDV as antigen. Virus neutralization test was performed using plaque reduction neutralization test using original US PEDV as antigen. The serum ELISA IgG-, IgA- and serum neutralization-titers were similar between the S-INDEL and virulent PEDV inoculated groups at post inoculation days (PID) 7, 14 and 21. No pigs in the S-INDEL- and virulent-groups developed diarrhea, whereas 100% pigs in mock group had diarrhea post-challenge. Serum neutralization titers at PID 21 correlated with the reduction in the virus shedding post-challenge with original virulent PEDV (correlation= -0.8 between virus neutralization titer and virus shedding with p=0.001). The number of antibody secreting cells (enumerated by ELISPOT) in the ileum was similar in the S-INDEL and virulent inoculated pigs pre-challenge and post-challenge. The sow milk neutralization antibody titers and isotype antibody titers of the S-INDEL and original PEDV inoculated groups were also similar. We conclude that the S-INDEL strain induces similar immune responses to the original PEDV strain that effectively cross protects against clinical disease by virulent strain.

057P

Virulence of Porcine Epidemic Diarrhea virus (PEDV) for weaning-age pigs

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A study was conducted to evaluate the virulence of 2 isolates of PEDV when administered to weaning-age pigs (Data on File, Study Report No. B82W-US-14-279, Zoetis Inc). Pigs, sourced from a farm free of PEDV, were randomly allotted to seven treatment groups; each group consisted of 10 piglets. One treatment group was not challenged (T01) and the others were challenged via esophageal gavage with 10<sup>3</sup> (T02), 10<sup>4</sup> (T03), or 10<sup>5</sup> (T04) TCID<sub>50</sub> per 10 mL of Isolate #1 or 10<sup>3</sup> (T05), 10<sup>4</sup> (T06), or 10<sup>5</sup> (T07) TCID<sub>50</sub> per 10 mL of Isolate #3. Clinical observations and fecal swabs were collected daily post-challenge and pigs were euthanized and necropsied 7 days post-challenge. Fecal swabs were analyzed for PEDV nucleic acid via RT-qPCR. Intestinal tissues were submitted to the Iowa State University Veterinary Diagnostic Laboratory, Ames, IA, and

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examined for villous atrophy and for PEDV staining via IHC. The study was valid in that the T01 control pigs remained negative for PEDV-associated clinical signs, intestinal lesions and fecal shedding. Pigs across all PEDV challenge groups showed clinical signs and had lesions consistent with PEDV infection (Table 1). Clinical signs were observed beginning 3 days post-challenge and continued until necropsy. Fecal shedding of PEDV was detected as early as Day 2 post-challenge in pigs inoculated with the higher doses (T03, T04, T06 and T07) and on Day 5 or 6 in pigs challenged with the lower doses (T02 and T05). Fecal shedding in high copy numbers was still apparent in all groups at necropsy on Day 7. Microscopic and macroscopic lesions associated with PEDV were present in pigs in all challenged groups. Under the conditions of this study, neither field isolate produced mortality in weaned pigs; however, both isolates, at all doses tested, induced clinical signs, intestinal lesions and persistent fecal shedding of the virus. In vivo procedures occurred according to state, national, or international regulations and after ethical review by Zoetis's IACUC.

058P

A comparison of serological tests for *Lawsonia intracellularis* in swine

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Porcine Proliferative Enteropathy (PPE) is a common and economically important gastro-intestinal disease of swine caused by the intracellular bacterium, *Lawsonia intracellularis*. Conventionally, the immuno - peroxidase monolayer assay (IPMA) is considered the gold-standard test. However, due to the difficulty associated with culturing *L. intracellularis* the IPMA is laborious to perform. Therefore, the performance of a commercial Enzyme-Linked Immunosorbent Assay (ELISA) for *L. intracellularis* was evaluated in this study. The commercial ELISA, IPMA and a non-commercial lipopolysaccharide (LPS) LPS-ELISA showed a 95% correlation when tested using experimentally derived known status samples (N=60). When the IPMA was used as the gold standard, the sensitivity of the commercial ELISA was 91% while the specificity was 100%. Therefore, the diagnostic sensitivity and specificity of the commercial *L. intracellularis* ELISA was comparable to the LPS-ELISA and IPMA. A comparison of suspect and randomly selected field samples with the commercial ELISA indicated that *L. intracellularis* sero-positivity is widespread and does not correlate with possible disease status.

059P

Comparative analysis of IS1096- and Himar1- derived transposon insertion sites in *Mycobacterium avium* subsp. *paratuberculosis*.

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Purpose: *Mycobacterium avium* subsp. *paratuberculosis* (MAP) is the etiologic agent of Johne's disease in ruminants and has been implicated as causing Crohn's disease in humans. The generation of comprehensive random mutant banks by transposon mutagenesis is a fundamental wide genomic technology to determine the role of MAP genes in pathogenesis.

Methods: In this study, bioinformatics was applied to perform a comparative analysis of insertion sites for the classical mycobacterial transposons Tn5367 and Tn5370, both derived from the *Mycobacterium smegmatis* insertion sequence IS1096 and the mariner transposons carrying the *Himar1* transposase.

Results: We have confirmed that only mariner transposons provide a random representation of insertions in almost any MAP gene. Further analysis of transposon recognition sites indicated that 16.3% (710) and only 0.85% (37) of all 4,350 MAP genes do not possess an IS1096 and *Himar1* insertion site, respectively. Thus, a significant number of MAP genes remain underrepresented in Tn5367 or Tn5370 insertion libraries. Experimental confirmation was provided by sequencing a subset of 30 *Himar1* mutants from a small pool of 109. Two *Himar1* insertion mutants were identified that lacked IS1096 insertion sites, while all 30 mutants had insertions in different MAP genes.

Conclusions: In summary, *Himar1* transposon mutants are necessary to obtain random mutant bank collections that could be used in the study of absolute and conditional (e.g., *in vivo*) gene essentiality.

060P

Establishment and characterization of primary and immortalized bovine ileal epithelial cell lines from a young calf

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The intestinal epithelial cells interact with enteric pathogens and play important role in mediating mucosal immune responses. No bovine intestinal epithelial cultures from young calves are available. Earlier we established bovine ileal cultures from a young calf which had both intestinal myofibroblasts and epithelial cells. The major goal of this study is to establish pure primary cultures of bovine ileal epithelial cells (BIECs) and then immortalize these cultures for developing continuous cell lines for studying their role in innate immunity to gut pathogens. To achieve this objective, bovine mixed ileal cultures were cultured in cell culture media which had been successfully used for culturing porcine intestinal epithelial (IPEC-J2) cells. Limiting dilution method was used to obtain a clone of ileal epithelial cells which was further amplified and characterized using immunohistochemistry. The selected clone, designated as C4, was cytokeratin positive and expressed low levels of vimentin. It was also negative for desmin and alpha-smooth muscle actin, confirming the epithelial cell phenotype of these cells. Immortalization of these cells was carried out with simian virus 40 large T antigen (SV40) plasmid and subsequent selection of G418 antibiotic resistant cells. The presence of SV40 gene in immortalized BIECs was confirmed by PCR. Immunofluorescence and immunohistochemistry assays also confirmed the expression of SV40 protein in these cells. The SV40 immortalized BIECs were also cytokeratin positive and vimentin negative confirming their epithelial phenotype. Primary BIEC cultures were also successfully immortalized using human telomerase reverse transcriptase (hTERT) gene. In future studies, growth kinetics of primary, SV40 and hTERT immortalized BIECs will be compared. Expression of various pattern recognition receptors (PRRs) genes in these cell lines will be assessed by real-time RT-PCR, and select PRR proteins will be detected by immunohistochemistry or flow cytometry. The successful establishment of these primary and immortalized BIECs will serve as a model system for studying the role of intestinal epithelial cells in mucosal immunity to enteric pathogens.



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062P

Evaluation of conjugative transfer of antimicrobial resistance and virulence among multidrug resistant and pansusceptible *Salmonella* from the food-animal industry

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The objective of this study was to evaluate the association between multiple-drug resistance (MDR) and virulence in multiple-drug resistant *Salmonella* (MDRS) and pansusceptible *Salmonella* (PSS) from different sectors of food-animal industry. *Salmonella* serovars originated from beef and dairy cattle, and animal feed sources in the United States. PCR-based replicon typing was used to characterize incompatibility plasmid replicons. PCR was done to detect antimicrobial and virulence genes. Conjugation experiments were done at 37°C to determine transferability of resistance and virulence traits. MDRS ( $n=100$ ) and PSS ( $n=100$ ) isolates were used as donors and recipients in conjugation experiments, respectively. Forty-eight percent of MDRS produced transconjugants (trMDRS) using *E. coli* as a recipient. Ninety-three percent of the PSS isolates were able to acquire resistance from an *E. coli* donor (trPSS). All trPSS acquired the exact antimicrobial resistance profile of the *E. coli* donor. Forty-one of the trMDRS displayed an identical resistance profile as the donor. Plasmid-mediated virulence genes, *spvC* and *rck*, were only detected in ten and five MDRS isolates, respectively. In contrast, *spvC* and *rck* were detected in 27 and one of the PSS isolates, respectively. Among the 48 conjugative MDRS both virulence genes (*spvC* and *rck*) were transferred to one transconjugant. Among the 27 *spvA* positive PSS, 25 were able to acquire resistance plasmids. The following plasmid replicons were detected among MDRS and (trMDRS): A/C, 55 (9); II, 24 (11); N, 10 (9); FIA, 1(0); FIB, 3 (1); HI1, 18 (14); P, 3 (1); X 1 (0); B/O, 12 (8); W 1 (0); HI1 5, (1). Although the incidence of virulence genes was low, there was no statistical significance between the transfer of virulence and resistance genes in the MDRS isolates at ( $p<0.05$ ). This suggests the plasmid-associated virulence genes present among these isolates were not located on the resistance plasmids. The greatest risk of enhanced association of antimicrobial resistance and virulence may result from the acquisition of resistance by pansusceptible *Salmonella* serovars that possess virulence traits.

063P

Development and evaluation of DNA vaccines for *Campylobacter* control in poultry

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Chicken is the major natural host of *Campylobacter*, the leading bacterial cause of human enteritis in the US and other developed countries. Thus, mitigation of *Campylobacter* in chicken using innovative strategies, such as vaccination, has a significant impact on food safety and public health. DNA vaccine has appeared to offer various advantages for poultry, particularly when combined with *in ovo* vaccination. Our previous studies have demonstrated that the two outer membrane proteins, CmeC and CfrA, are feasible candidates for immune intervention against *Campylobacter*. Therefore, in this study, to develop effective DNA vaccines, *cmeC* or *cfrA* genes were cloned into eukaryotic expression vector pCAGGS with introduction of Kozak sequence to further enhance the production level of inserted genes in eukaryotic cells. Two independent *in ovo* vaccination trials have been performed to evaluate the immune response and protective efficacy of the validated DNA vaccines. We observed that *in ovo* injection of original DNA vaccines at 18<sup>th</sup> day of embryonation failed to trigger significant immune response in broilers, likely due to the presence of DNase in amniotic fluid. The *in ovo* delivery of the DNA vaccines with neutral lipid encapsulation DNA vaccines was evaluated for enhanced stability of DNA and *in vivo* expression of desired antigen. This study clearly demonstrated that introduction of the Kozak consensus sequence is critical for constructing DNA vaccines expressing bacterial antigens. In addition, the *in ovo* vaccination regimen for the constructed DNA vaccines should be further optimized to trigger sufficient protective immunity in poultry.

064P

Identification of the Factors Required for High Frequency Conjugation in *Campylobacter jejuni*

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*Campylobacter jejuni*, the leading bacterial cause of human gastroenteritis in the United States, displays significant strain diversity due to horizontal gene transfer. Conjugation is an important mechanism for horizontal gene transfer. It has been observed that conjugation efficiency varies significant in various *C. jejuni* strains. The underlying genetic components contributing to high frequency conjugation (HFC) *C. jejuni* are still unknown. In this study, bi-parental conjugation was performed for diverse *C. jejuni* strains, which confirmed significant conjugation efficiency difference in *C. jejuni* with an extremely high efficiency for JL686 ( $1 \times 10^{-4}$  CFU/recipient cell) and low efficiency for NCTC 11168 ( $1 \times 10^{-8}$  CFU/recipient cell). To examine molecular basis of conjugation, the *chuB::erm* was first introduced into *C. jejuni* JL686 and the *erm* cassette served as a feasible marker to identify genetic loci required for HFC using a unique co-transformation selection strategy. The putative HFC factors in *C. jejuni* JL686 were naturally transformed into *C. jejuni* NCTC 11168 with enrichment and selection by the *erm* marker. Total 9 NC11168 derivatives displaying HFC phenotype were identified and were further confirmed by plasmid cure. Six HFC derivatives and two low frequency derivatives were subjected to whole genome sequencing using MiSeq. The genome sequences were used for comparative genomics analysis to identify genetic components contributing to HFC in *C. jejuni*. Together, this study successfully developed a unique co-transformation strategy to identify factors required for HFC in *C. jejuni*, which has established a solid foundation for us to elucidate molecular mechanisms of conjugative gene transfer in the future.

065P

Evaluation of passive immunotherapeutic efficacy of hyperimmunized egg-yolk powder against intestinal colonization of *campylobacter jejuni* in chickens

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Purpose: *Campylobacter jejuni* is a leading cause of food-borne bacterial gastroenteritis in human. Chickens are the reservoir host of *C. jejuni* and contaminated chicken meat is an important source of human infection. Therefore, control of *C. jejuni* in chickens can have direct impact on human health. The purpose of this study was to test the passive immunotherapeutic efficacy of the chicken egg-yolk-derived antibodies, in the

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form of hyperimmunized egg-yolk powder (HEYPs), against seven colonization associated proteins of *C. jejuni* namely: CadF, FlaA, MOMP, FlpA, CmeC, Peb1A, and JlpA.

Methods: Three chicken experiments were performed. In each experiment, chickens were treated orally via feed supplemented with 10% (w/w) egg-yolk powder. In experiment-1, chicken groups were experimentally infected with *C. jejuni* ( $10^8$  CFU) followed by treatment with five HEYPs (CadF, FlaA, MOMP, FlpA, CmeC) for 4 days either individually or as a cocktail containing equal part of each HEYP. In experiment-2, chickens were treated for 21 days with cocktail containing equal parts of seven HEYPs before and after experimental infection with *C. jejuni* ( $10^8$  CFU). In experiment-3, chickens were treated with feed containing a cocktail of seven HEYPs before and after (prophylaxis), and after (treatment) experimental infection with *C. jejuni* ( $10^5$  CFU). Intestinal colonization of *C. jejuni* was monitored by culturing cecal samples from chickens euthanized at the end of each experiment.

Results and Conclusions: The results showed that there were no differences in the cecal colonization of *C. jejuni* between HEYP treated and non-treated control chickens. The use of HEYP at the dose and the regimes used in current study is not efficacious in reducing *C. jejuni* colonization in chickens.

066P

Biofilm formation and antibiotic resistance among most prevalent poultry *Salmonella* serotypes isolated from US poultry

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*Salmonella* contaminated poultry meat and eggs are the major sources of infection for human salmonellosis. Antibiotic resistance among poultry-associated *Salmonella* poses a significant public

health concern. *Salmonella* also produces biofilm on food and food-processing surfaces which may further aid in resistance to antibiotics, disinfectants and various sanitizers during poultry meat processing. The objective of this study was to determine the antimicrobial resistance and the biofilm forming ability of most prevalent poultry-associated *Salmonella* serotypes (MPPSTs) isolated specifically from US poultry. A total 139 MPPSTs isolated from US poultry including *S. Kentucky* (53), *Enteritidis* (41), *Typhimurium* (14), *Heidelberg* (12), *Mbandaka* (9), *Seftenberg* (4) and three strains each of *Montevideo* and *Infantis* were tested for their ability to form biofilms and susceptibility to 12 antibiotics belonging to penicillin, quinolone, cephalosporin, aminoglycoside, sulfonamide and tetracycline classes. A total of 35 of 139 (25%) isolates formed biofilm on polypropylene surface. The proportion of biofilm forming strains was higher among serotypes *Mbandaka* (44%, 4/9) followed by *Infantis* (33%, 1/3), *Enteritidis* (27%, 11/41), *Kentucky* (25%, 13/53) and *Heidelberg* (17%, 2/12). All isolates were susceptible to amikacin, sulfamethoxazole/trimethoprim and ciprofloxacin. Overall, 120 of 135 (86%) isolates were resistant to at least one antibiotic. Resistance to sulphisoxazole was dominant (93%, 111/120). Among the MPPSTs, 62% of *Kentucky* (33/53) were multidrug resistant ( $\geq 3$  up to 6 antibiotics) followed by *Heidelberg* (42%, 5/12), *Typhimurium* (21%, 3/14) and *Enteritidis* (2%, 1/41). Resistance to ceftiofur, a third generation cephalosporin was observed in 6% (3/53) of *Kentucky*. One *S. Typhimurium* and *Enteritidis* isolate was resistant to Nalidixic acid. Multidrug resistance among MPPSTs is common. Several *Salmonella* strains produce biofilm which could further enhance resistance to antibiotics and also to the carcass sanitizers or disinfectants used during meat processing. Further studies are needed to investigate the role of biofilm in antimicrobial resistance among MPPSTs.

Conclusions: The results of this study prove that MALDI-TOF MS is a viable strategy for the identification and characterization of AMR bacteria associated with CAFOs. However, the method is limited in that relatively few reference spectra from animal and agriculturally-relevant bacterial isolates are currently available in commercial spectral databases.

## Respiratory Diseases Posters

067P

Diagnostic investigation of an outbreak of respiratory and reproductive disorder in a Polish pig farm.

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PRRSV is a viral pathogen associated with respiratory and reproductive disorders. Acute infection with IAV may too induce reproductive disorder. As both pathogens often co-circulate in farms and clinical symptoms of both infections can be similar, differential diagnosis of respiratory and reproductive failure can be challenging.

In February 2013 a Polish, two site pig farm with 400 sows, positive for PRRSV and negative for IAV, experienced acute respiratory disorder in sows and all age groups of pigs as well as reproductive failure in sows. Laboratory diagnostic investigation confirmed acute PRRS outbreak caused by PRRSV Type 1. The disease control program was implemented that involved Porcilis PRRS (MSD) vaccination and the clinical situation significantly improved in the following months. In October 2013 respiratory and reproductive disorder re-appeared in the farm. Percentage of stillborn increased from 5.5% in August and September, to 7.38 % in October, 6.33% in November and 7% in December 2013. Also proportion of mummies increased from below 1% in October to 2.77% in December.

In the beginning of March 2014, samples of lungs from three suckling piglets were collected, as well as oral fluid samples from pens of 7 weeks old weaners and 10, 13, 16, 19 and 24 weeks old fatteners. Influenza virus was detected by PCR (Universal Influenza MPX Real-Time PCR, Tetracore) in lung samples from piglets as well as in oral fluid samples from 7, 16 and 19 weeks old pigs. Of those samples only oral fluid and serum from 10 weeks old fatteners tested positive in PCR for PRRSV (EZ-PRRSV™ MPX 4.0, Tetracore). The PRRSV seroconversion was also confirmed only in fatteners with the IDEXX PRRS X3 Ab Test.

These results indicate that the outbreak of respiratory and reproductive disorder that started in October 2013 was caused by infection of previously naïve farm with IAV. Interestingly, the most acute respiratory disease was observed in fatteners where PRRSV and IAV co-circulated. Oral fluid is a good alternative to serum as it allows simultaneous detection of several pathogens present in the respiratory tract or the environment.

## Respiratory Diseases Posters

068P

Simultaneous detection of PRRSV and SIV by a one-step triplex real time RT-PCR assay

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Porcine Reproductive and Respiratory Syndrome (PRRS) and Swine Influenza (SI) are two important infectious diseases. PRRSV has two distinct genotypes: the North American type (NA) and the European type (EU). The three dominant influenza virus subtypes currently circulating in swine populations are: H1N1, H3N2, and H1N2. Pigs, especially piglets, are often co-infected with PRRSV and SIV and showing similar clinical signs, making it difficult for accurate clinical diagnosis. We have developed a one-step triplex real time PCR (qPCR) for rapid, simultaneous detection and differentiation of PRRSV-NA, PRRSV-EU and SIV. The most conserved region in the N gene of PRRSV and M gene of SIV were selected as detection targets for qPCR assay development. Our results showed that the multiplex assay generated similar sensitivity levels as compared to its corresponding single-target PCRs. The amplification efficiencies of multiplex qPCRs were 92.5%, 99.9% and 105.3%, and the correlation coefficients of Ct values from the standard curves generated by the multiplex reaction and its corresponding singular reactions were 0.9989, 0.9997 and 0.9994, for PRRSV-NA, PRRSV-EU and SIV, respectively. The detection limits of the triplex qPCR were around 10 copies per reaction for the three viruses, which are comparable to its corresponding singular qPCR reactions. When primers and probes of the three viruses were used in the same reaction on individual virus template, only the corresponding channel generated signal, and there is no cross-reaction or interference observed. Similar specificity was also obtained from singular reactions. Testing of 80 clinical serum samples revealed that 43 were positive for PRRSV-NA, 1 was positive for SIV, 1 was positive for PRRSV-EU, and 5 were co-infected with PRRSV-NA and SIV. There were 53 lung tissues were tested, of which 28 were positive for SIV, 3 were positive for PRRSV-NA and 5 tissues were co-infected with SIV and PRRSV-NA. Taken together, our results indicate that this one-step triplex real time RT-PCR assay can simultaneously detect PRRSV and SIV in one clinical sample.

069P

Comparative analysis of routes of immunization of a live PRRS virus vaccine in a heterologous virus challenge study

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Purpose: Porcine reproductive and respiratory syndrome (PRRS) has been an economically important disease since 1991. Effective mucosal vaccination induces both mucosal and systemic immunity compared to parenteral vaccination. PRRSV causes disease primarily in the respiratory tract and thus intranasal (IN) delivery of a potent vaccine adjuvant formulation has a great promise.

Methods: PRRS-MLV (Boehringer Ingelheim) (strain VR2332) was coadministered with an adjuvant *Mycobacterium vaccae* whole cell lysate (*M.vaccae* WCL) or CpG ODN, intramuscular (IM) or IN route (as an aerosol), followed by a heterologous PRRSV (strain 1-4-4) challenge at day post-vaccination (DPV) 42 by IN route. Viral load and immune correlates were determined in the blood, lungs, and tonsils.

Results: At DPV 14, 26, and 42 the vaccine virus RNA load was greater in the plasma of pigs received the vaccine through IM than IN route, but the data was not statistically significant; and the replicating virus was detected only in IM vaccinated groups. In vaccine +/- adjuvant received by IM route and virus challenged pigs at DPC 7, 10, and 14, reduced viral RNA load and absence of detectable replicating virus was detected compared to IN trial groups. Similarly, the viral RNA load in BAL fluid and the lungs of IM vaccine groups at DPC 14 was lower than IN trial groups, but in tonsils it was comparable. Immunologically, virus neutralizing antibody titer in the plasma and lungs of IM vaccine groups was higher than IN groups, but the data was not significant. Interestingly, at DPV 26 PRRS-MLV IM (without adjuvant) received pigs had significantly increased frequency of IFN- $\gamma$  secreting CD3<sup>+</sup>CD4<sup>+</sup>CD8<sup>+</sup> and CD3<sup>+</sup>CD4<sup>+</sup>CD8<sup>+</sup> lymphocytes in PBMCs stimulated with PRRSV or highly conserved pooled five T cell peptides of PRRSV. Furthermore, at DPC 14 the same pig group had significantly increased frequency of IFN- $\gamma$  secreting  $\gamma\delta$  T cells in virus stimulated PBMCs.

Conclusions: Our data suggested that PRRS-MLV administered by IM route induces the virus specific T-cell response and reduces the challenge virus load. However, as reported earlier potent adjuvant/s like *M. tuberculosis* WCL significantly augment the cross-protective response of the PRRS-MLV.

070P

Quantification of UV-induced RNA damage of porcine respiratory and reproductive syndrome virus

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Purpose: In order to prevent economic losses from PRRS, many swine producers use UV light as a sterilizer for the workers, equipment, surface of farm units etc. However, it is very little known about its actual degree of UV-induced RNA damage and effectiveness. The aim of the experiment was to measure levels of UV-induced RNA damage by utilizing the property that damaged RNA of PRRSV can inhibit PCR.

Methods: The PRRS virus strain, ATCC VR2332, was used to assess the analytical performance of the reverse transcriptase PCR (RT-PCR) and real time-PCR (ReTi-PCR) protocols. This virus was treated by UV ramp (Enputech, Korea) with wavelength output at 254nm, 150 $\mu$ W $\cdot$ sec/cm<sup>2</sup>, for 0.5, 1, 2, 4, 8, 16, and 32 min, respectively. RT-PCR amplified a 100bp (1154-1253) region (Table. 1) and the amplicons were diluted 10<sup>-5</sup>-fold and analyzed by using SYBR<sup>®</sup> Green I Method for ReTi-PCR.

To evaluate effects of UV induced RNA damage, Regression normalization (a linear trendline correction) was used.

Table 1. Oligonucleotide primer

Results: The inactivation ratio of PRRSV was relatively quantified results from ReTi-PCR. The Ct value of serially diluted positive control sample showed the linear correlation ( $R^2=0.999$ ) (Fig. 1).

Fig 1. Linear trendline correction of standard curve of serially diluted control samples

Y axis is Ct value and X axis is -Log(dilution ratio)-1.

The inactivation of PRRSV by UV light was dose dependent (Table. 2).

Table 2. The result of Ct value and inactivation ratio by exposure time using ReTi-PCR.

In the setup of the experiment, 1D, 2D, and 3D (D=1log) inactivation were found 10.72, 72, and 638.2mJ/cm<sup>2</sup>, respectively.

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Conclusions: UV is cost effective and environment friendly for disinfection of PRRSV in pig farm units. It is thought that these results can be a useful data for sterilizing of PRRSV.

071P

Effect of bovine viral diarrhea virus (BVDV) infection on neutrophil survival and surface marker expression

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Neutrophils are the predominant white blood cells in peripheral blood and play an important role against invading bacteria. The ability of neutrophils to migrate or destroy the invading microorganism depends on surface marker expression. The neutrophil surface markers CD14, CD18 and L-selectin play an important role in bacterial recognition, neutrophil binding and migration. Viruses that affect the neutrophil viability or surface marker expression may significantly affect the immune defense. In the current study, the effect of bovine viral diarrhea virus (BVDV) on bovine neutrophils viability and surface marker expression was investigated. The neutrophils were isolated from bovine peripheral blood and confirmed phenotypically as CD14+, CD18+, and L-selectin+. Isolated neutrophils were infected with either BVDV [e.g. BVDV-1b cytopathic (cp) TGAC or BVDV-1b non-cytopathic (ncp) TGAN] at 3 M.O.I., while lipopolysaccharide (LPS), 10 ng/ml was used as the positive control. Neutrophils were examined for apoptosis and cell surface expression at 1 hr and 6 hrs post infection (PI). Both BVDV biotypes induced apoptosis in neutrophils. The TGAC induced comparatively more apoptosis than TGAN at 1 and 6 hrs PI. BVDV infection with either biotype had little effect on selectin compared with LPS, which down regulated the L-selectin. LPS significantly down regulated the L-selectin at 6 hrs PI ( $p < 0.05$ ). There was a slight reduction in CD14 expression by cp TGAC at 1 hr and 6 hr PI, while ncp TGAN down regulated the CD14 at 1 hr PI followed by a slight up regulation at 6 hrs PI. LPS upregulated the CD14 expression during treatment with a significant increase at 6 hrs PI ( $p < 0.05$ ). CD18 was down regulated at 1 hr PI followed by slight up regulation at 6 hrs PI by both biotypes. In contrast, LPS significantly down regulated the CD18 during the course of treatment ( $p < 0.05$ ). In summary, BVDV infection resulted in increased apoptosis but had less effect on surface marker expression than the LPS control. Further studies need to be done to determine the effect of more virulent strains on these parameters as well as measuring the effect of these strains on neutrophil migration and oxidative burst.

072P

Development of automated DNA microarray based assays for multiplex detection of bovine pathogens

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Respiratory and enteric diseases are the most common and costly infectious diseases in beef cattle. Bovine respiratory disease (BRD) is a multifactorial disease complex involving several viruses and bacteria. BRD accounts for 50-70% of feedlot deaths and costs over \$1 billion in annual losses to the beef industry in North America. Enteric diseases are the second most costly diseases in beef cattle. About 5% of all beef calves die of scours and these deaths are mostly due to viral, bacterial and/or protozoan pathogens. Effective control of these diseases, often caused by multiple pathogens, can benefit from rapid and cost-effective multiplex diagnostic tests that can simultaneously detect all relevant pathogens in a single assay. Current diagnostic tests for these diseases, however, are primarily single pathogen tests and are thus inefficient and require separate tests for each pathogen. DNA microarray, when combined with multiplex PCR, is capable of highly sensitive detection and differentiation of multiple pathogens in a single sample. The objective of this study is to develop two bovine microarray chips; one for rapid identification of BRD pathogens and the other one for enteric pathogens of beef cattle. Using available sequence data, we created a large sequence database of the targeted viral, bacterial and protozoan pathogens, and the database was used to design primers and probes specific for each pathogen species. Here we describe development of multiplex PCR assays for main viral, bacterial and mycoplasma agents of BRD and main viral, bacterial and protozoan agents of bovine enteric diseases. The multiplex PCR assays will be combined with a user-friendly automated microarray platform and validated with laboratory and clinical samples.

073P

Efficacy test of broad spectrum Canine Influenza Vaccine in guinea pigs

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Influenza virus occurs antigenic shift and drift giving a variety of viral strains, which is challenging to prepare for mutated antigenic proteins. To get through its dynamic changes, it is important to utilize conserved regions of influenza antigenic proteins for developing broad-spectrum influenza vaccines. Recently, hemagglutinin 2 (HA2) protein, stem region of influenza HA protein, comes into the spotlight due to its conserved feature. We thought that the notable characteristic of HA2 protein could be exploited as a broad range influenza vaccine that covers variety of influenza viruses for hosts such as dogs that are vulnerable to different HA groups 1 and 2 influenza viruses; pandemic H1N1 and canine H3N2 respectively. Here, we designed stalk domain HA vaccine from H1N1, and studied its vaccine efficacy against H1N1 and H3N2 canine influenza virus (CIV) challenges. Trimerized antigenic protein was expressed from hemagglutinin stalk domain (HA2) to be utilized as vaccine; 3xHA95. To evaluate its application to broad-spectrum vaccine, we vaccinated 3xHA95 into guinea-pig as previously described and challenged with H3N2 CIV. During experiment, nasal swabs were collected daily during the experiment to confirm viral shedding by real-time RT-PCR (rRT-PCR). We found that the 3xHA95, originated from PR8, produced a protective vaccine efficacy showing less body weight change and enhancing survival rate in mice challenged with PR8. Also 3xHA95 vaccination shortened the duration of viral shedding in guinea-pig challenged with canine H3N2. Here, we successfully purified partial antigenic HA stalk domain protein from PR8 and conducted its vaccine efficacy study for influenza virus challenges in mice and guinea-pigs. In vaccinated animals, protective vaccine efficacy was observed against PR8 and H3N2 CIV, which belongs in different HA group 1 and 2 respectively. Our results are meaningful for further understanding of an applicable range of the purified HA stalk domain protein and development of broad-spectrum vaccines, and this evokes further studies in dogs which is co-infected with H3N2 CIV and H1N1 influenza virus.

## Vector-Borne and Parasitic Diseases Posters

074P

Investigating white-tailed deer as a potential amplifying host for *Borrelia miyamotoi*, a relapsing fever group spirochete transmitted by blacklegged ticks.

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*Borrelia miyamotoi* was first detected in North America in 2001 and recently has been implicated as a human pathogen. In the eastern United States, *B. miyamotoi* is transmitted by the blacklegged tick, *Ixodes scapularis*, which also vectors *B. burgdorferi*, the etiologic agent for Lyme disease. *Borrelia miyamotoi* has been detected in ticks, blood, or tissue of rodents and birds, but the key reservoir host(s) remains uncertain. Prompted by preliminary findings of sporadically high prevalences of *B. miyamotoi*-infected ticks collected from white-tailed deer, we investigated the hypothesis that deer may serve as a reservoir for *B. miyamotoi* by comparing the infection prevalence of questing adult ticks and those attached to deer.

From 2010-2012 we collected 730 questing adult ticks at Fort McCoy, Wisconsin, where blacklegged ticks and associated pathogens are highly abundant. In 2010 we removed 355 adult ticks from hunter-harvested deer from the same area. We screened the total DNA of each tick for the presence of *Borrelia* spp. by targeting a fragment of the 16S rDNA of *Borrelia* spp. If deer were to amplify infection, we predicted that the infection prevalence of deer-associated adults would be greater than that in questing adults.

As predicted, there was a significant increase in infection prevalence between questing (0.82%) and deer-associated (4.51%) adults ( $p = 0.0001$ , Fisher's exact test). Furthermore, the infection prevalence of deer-fed (7.05%) females was the greatest and was 11.2 times greater than that of questing females (0.63%) ( $p = 0.0001$ , Fisher's exact test). These results support our hypothesis that feeding on white-tailed deer amplifies *B. miyamotoi* prevalence among the adult *I. scapularis* population.

The results suggest that deer may contribute to the disease ecology of *B. miyamotoi* beyond serving as the main reproductive host of adult *I. scapularis*. Future research should investigate by xenodiagnosis the extent to which deer amplify transmission to other tick life stages. Using these findings, mathematical modeling could help assess whether deer are sufficient to maintain *B. miyamotoi* in nature or whether contributions from additional competent hosts are also necessary.

## Viral Pathogenesis Posters

075P

Assessing the role of PRRSV envelope minor glycoprotein 3 in the induction of an immune response in swine using a heterologous prime-boost vaccination schedule

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The role of PRRSV minor envelope glycoproteins in the induction of a protective immune response in swine is an area of research that may have a major impact on the development of a broadly protective vaccine. The objective of this study is to assess the contribution of GP3, a minor glycoprotein present on the surface of the PRRSV envelope, to the induction of an immune response in pigs. To accomplish this, a prime-boost vaccination schedule using heterologous vector systems is currently being tested in animals. We elected to use an adenovirus vector as a gene delivery system and a baculovirus vector for recombinant protein production. GP3 was cloned in to the Invitrogen ViraPower Adenoviral Expression System and the Invitrogen Bac-to-Bac Baculovirus Expression Vector System, and protein expression was confirmed by western blot. Virus and protein production were optimized and scaled up for use in animal studies. Adenovirus vectored GP3 served as a priming vaccination; then animals were boosted with recombinant protein produced in a baculovirus expression vector system. Antibody responses will be evaluated by ELISA and neutralization assays, and animals will be challenged three weeks after boost to evaluate protection.

076P

Mutations in the highly conserved GKYLQRRLLQ motif of nsp1 $\beta$  protein impairs the innate immune suppression function of porcine reproductive and respiratory syndrome virus (PRRSV)

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The PRRSV nonstructural protein 1 $\beta$  (nsp1 $\beta$ ) has been identified to be a strong innate immune antagonist. Recently, this protein was determined to function as a transactivator for expression of -2/-1 programmed ribosomal frameshifting (PRF) products, nsp2TF and nsp2N. Both nsp2TF and nsp2N contain a papain-like protease domain (PLP2) that has been implicated in disrupting innate immune signaling by acting as a deubiquitinating enzyme, which is associated with innate immune suppression function of the virus. Embedded in nsp1 $\beta$ 's papain-like autoprotease domain (PLP1 $\beta$ ), we identified a highly conserved GKYLQRRLLQ motif that is critical for PRF transactivation and innate immune suppression function of the virus. In this study, we further investigated the function of three basic residues (K124, R128, R129) in GKYLQRRLLQ motif that are exposed on the surface of the protein. Site-directed mutagenesis analysis showed that R128A or R129A mutation impaired PRF transactivation function of nsp1 $\beta$ , as well as reduced the ability of nsp1 $\beta$  to suppress IFN- $\beta$  and reporter gene expression. However, only R128A mutation affects the ability of nsp1 $\beta$  to suppress "self-expression" in vitro. Subsequently, three viable recombinant viruses, vSD95-21-R128A, vSD95-21-R129A and vSD95-21-R128R129AA, carrying single or double mutations in the GKYLQRRLLQ motif, were created using reverse genetics. In comparison to the wild-type virus, vSD95-21-R128A and vSD95-21-R129A show similar growth ability, while vSD95-21-R128R129AA mutant had slightly reduced growth ability with about 0.5 log<sub>10</sub> decrease in viral titer in MARC-145 cell. When tested in porcine alveolar macrophages, a 5-10 fold higher level of IFN- $\alpha$  and ISG15 expression were detected in mutant-infected macrophages comparing to wild type virus-infected macrophages. The result demonstrates that R128 and R129 residues are critical for nsp1 $\beta$  function, and modifying these key residues in the GKYLQRRLLQ motif could attenuate virus growth and improve the cellular innate immune responses.

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077P

Attenuation of porcine reproductive and respiratory syndrome virus (PRRSV) by inactivating the expression of ribosomal frameshifting products nsp2TF and nsp2N

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The ribosomal frameshift products of PRRSV, nsp2TF and nsp2N, are novel proteins recently identified. In this study, they were determined having ability to interfere with cellular protein ubiquitination (Ub) and ISGylation activities that associated with innate immune suppression function of the virus. Two recombinant viruses (KO1 and KO2), generated by partially or completely inactivation of nsp2TF/nsp2N expression, displayed impaired de-Ub and de-ISGylation ability and induced higher levels of IFN- $\alpha$  and ISG15 expression in infected cells. When tested in a nursery pig model, these mutants showed reduced level of viremia compared to wild-type (WT) virus. After challenge, mutant virus-immunized pigs demonstrated improved protection with reduced lung lesion and viral load in lung and tonsil tissues, in comparison to pigs immunized with WT virus. Our data strongly implicate PRRSV nsp2TF/nsp2N in viral immune evasion and demonstrate that nsp2TF/nsp2N-deficient viruses are attenuated *in vivo*. Thus, manipulation of nsp2TF/nsp2N expression may be used in the rational design of improved PRRSV vaccines.

078P

In vitro inhibition of porcine reproductive and respiratory syndrome virus (PRRSV) replication by specific DNA aptamers

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Porcine reproductive and respiratory syndrome (PRRS) is the viral disease with the highest economic impact on swine production in North America. PRRS is caused by an enveloped, single-stranded RNA virus (PRRSV) belonging to *Arteriviridae* viral family. PRRSV cause reproductive failure and increased mortality in young pigs as a result of severe respiratory disease and poor growth performance. Currently, vaccination is the principal available measure to control and prevent the disease. However, the success obtained with vaccines is rather mitigated. Therefore, finding new, safe, effective and inexpensive ways to control PRRSV infection is imperative. Aptamers are a new class of therapeutic molecules composed of synthetic nucleic acid capable of binding to a broad range of targets with high affinity and specificity. Aptamers has the potential to inhibit viral infection at any stage in the viral cycle, including viral entry. The objective of this study was to select PRRSV-specific DNA aptamers and evaluate their antiviral capacity *in vitro* in MARC-145 and porcine alveolar macrophages (PAM) infected cells. Synthetic ssDNA library was used to select PRRSV-specific aptamers using systematic evolution of ligands by exponential enrichment (SELEX) technique. 6 candidate's aptamers were selected and evaluated for their binding capacity and their antiviral activity. Two candidate's aptamers (S16 and S18) had a significantly higher binding capacity to PRRSV. Only S18 aptamer significantly decreased the mortality caused by the homologous virus in MARC-145 cells, suggesting an antiviral effect of this aptamer. In addition, S18 also demonstrated a significant antiviral effect against a heterologous genotype II strain (IAF-Klop). Candidate aptamers had no effect on a heterologous genotype I strain (LV), showing genotype specificity. S18 aptamers significantly decreased the viral titers of homologous and heterologous virus in PAM cells. As in MARC-145 cells, candidate aptamers had no significant effect on genotype I LV strain. In conclusion, S18 aptamers showed some *in vitro* inhibitory effect on homologous and heterologous PRRSV genotype II strains, in MARC- 145 and PAM cells.

079P

Induction mechanisms of type-I IFN, inflammation, and apoptosis by H3N2 canine influenza virus in canine tracheal epithelial cells

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Mammalian host cells infected with influenza virus suppress replication of influenza virus through two main defense mechanisms. One mechanism is mediated by pattern recognition receptors which lead to the induction of NF- $\kappa$ B activation and production of type-I IFN through complex pathways after detection of viral RNAs. The other one is apoptotic response of host cells infected with virus. Purpose of our study is to identify expression pattern of TLRs and apoptotic genes of canine tracheal epithelial (KU-CBE) cells infected with canine influenza virus (CIV) and to determine which gene of influenza virus activates NF- $\kappa$ B signaling pathways. Expression patterns of TLR3, TLR7, TLR8, caspase-3, 8, 9, Bax, and Bcl-2 were measured by real-time PCR in the KU-CBE cells infected with 0.01 MOI of H3N2 CIV. Each of 8 genes of CIV was cloned into pcDNA 3.1 expression vector and was transfected into the KU-CBE cells. Then the cells were stimulated for 24 h with poly(I-C) and activation of NF- $\kappa$ B was determined by real-time PCR. When CIV infected the KU-CBE cells for 24 h, expression of TLR3 recognizing the viral dsRNA was significantly increased than that of mock infected cells. When vectors containing each of 8 CIV genes were transfected into the KU-CBE cells, the NS1 protein significantly induced expression of NF- $\kappa$ B gene than other genes. Expression of caspase-3, 9, and Bax mRNA increased in KU-CBE cells that were infected with H3N2 CIV for 2 days. These data indicate that KU-CBE cells infected with H3N2 CIV induce type-I IFN through TLR3-mediated signaling pathway. In addition, infection of H3N2 CIV in the KU-CBE cells leads to increased apoptosis. The NS1 protein mediates inflammation through activation of NF- $\kappa$ B.

080P

Serologic surveillance of antibody against seasonal human flu (H1N1 & H3N2) and canine flu (H3N8) viruses in dogs in Ohio

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Considering the devastating outcomes from spill-over infection and interspecies transmission of influenza virus, it is imperative to search potential hosts and understand their role. In last decade, two different lineages of influenza virus have successfully established in canine species and dogs have emerged as an important host for influenza. However, limited studies have been conducted to better understand the epidemiology of influenza virus in this species. We conducted a serologic surveillance of influenza infection in dogs to estimate the exposure to both human and canine influenza virus and to assess the risk factors. Eleven hundred and sixteen serum samples collected from domestic dogs in Ohio (from

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October 2012 to June 2014) were tested for antibodies against human seasonal influenza H1N1 (aka pandemic H1N1) and H3N2 and canine influenza (H3N8) viruses. In addition, the correlation of the prevalence with risk factors, such as season, age and the presence of respiratory symptom was evaluated. The prevalence of seasonal H1N1, H3N2, and canine H3N8 were 5.4%, 4.4 % and 2.4%, respectively. In this study, all samples were from household dogs which may explain the lower prevalence of canine influenza than previous report in shelter and racing dogs. The risk of dogs with clinical respiratory symptoms were observed to be about six times the risk of healthy dogs getting sero-positive result against seasonal H1N1 virus ( $p$ -value=0.0337). The prevalence was observed to be evenly distributed among different age groups with slightly higher prevalence of seasonal H1N1 in dogs older than 15-years-old. No significant correlation was observed between the prevalence and the season when the samples were collected. These findings highlights the susceptibility of dogs to human influenza virus and warrants extended monitoring of influenza activity in dogs for the implication of interspecies transmission in relation to both canine and human health.

081P

Measurement of tissue factor activity as a biomarker of infection of the spinal cord vasculature with equine herpesvirus-1

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Equine herpesvirus type 1 (EHV-1) causes respiratory disease, late-term abortions, neonatal foal death, chorioretinopathy and equine herpesvirus myeloencephalopathy (EHM) in horses worldwide. The development of EHM has a devastating impact in approximately 10% of infected horses. Our understanding of EHM pathogenesis is rudimentary beyond the importance of viremia for delivery of the virus to the spinal cord. This results in infection of the spinal cord vasculature and progressive damage of the microvessels due to inflammation, vasculitis, and microthrombosis. To further explore the events leading to EHM, we hypothesized that increased peripheral blood mononuclear cells (PBMC) expression of tissue factor (TF) activity, the primary activator of coagulation, may associate with viremia and the events leading to EHM.

Horses were experimentally infected with EHV-1 ( $n=8$ ) or remained uninfected ( $n=8$ ). Blood samples were collected prior to infection and daily for 10 days post infection (p.i.). PBMCs were isolated and TF activity was measured and correlated with clinical data and viremia following infection. PBMCs isolated from uninfected horses were also infected with EHV-1 in vitro or stimulated with lipopolysaccharide (LPS).

All infected animals developed signs of EHV-1 respiratory disease and showed a typical biphasic fever response. One horse developed mild ataxia on days 9-11 p.i. and two horses were euthanized on day 9 due to severe EHM. TF activity in equine PBMCs collected from EHV-1 infected horses was not induced significantly, while LPS stimulation or in vitro infection of PBMCs with EHV-1 resulted in significant TF induction.

Although the results indicate that EHV-1 induces TF activity in vitro, our results suggest that the measurement of PBMC TF activity is not a useful biomarker for predicting infection of the spinal cord vascular endothelium with EHV-1, or clinical EHM in vivo. This indicates that the mode of infection may be different in vivo and in vitro and likely affects the virus's ability to modulate responses of PBMCs. Furthermore the possibility that TF expression by other cell types contributes to EHM can not be excluded, and this is the focus of ongoing studies.

082P

Sensitivity improvement of pan-viral DNA array and high-throughput sequencing with propidium monoazide (PMA) for the identification of viruses from tissue samples

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Pan-viral DNA array (PVDA) and high-throughput sequencing (HTS) are useful tools to identify novel virus of emerging diseases. Although PVDA and HTS work well with isolated virus, they are less sensitive to detect viruses in tissue samples. This is because of host genomic DNA (hgDNA) contaminating nucleic extract from tissue samples. Both propidium monoazide (PMA) and ethidium bromide monoazide (EMA) have the capacity to bind free RNA and DNA but are cell membrane-impermeable and thus are unable to bind protected RNA/DNA such as virion protected viral genomic material. DNA permanently linked to PMA or EMA following photolysis is not amplifiable by RNA/DNA polymerase. Thus a PMA or EMA treatment before nucleic extraction could lower hgDNA contamination. To validate this hypothesis, lung tissue homogenates were spiked with porcine reproductive and respiratory virus (PRRSV) and were processed with different combination of treatment: with/without ultracentrifugation and incubation with/without different concentration of EMA or PMA. Following each treatment, total DNA/RNA was extracted. Quantitative PCR (qPCR) was used to evaluate hgDNA contamination (beta-actin) and PRRSV presence in each DNA/RNA sample. Finally, PVDA and HTS were used to detect PRRSV in each DNA/RNA samples. Both EMA and PMA treatment increased beta-actin quantification at least by  $11.40 \pm 0.52$  Ct ( $p < 0.001$ ), indicating an important loss of hgDNA contamination in these samples. While EMA caused a dose-dependent decrease of PRRSV qPCR detection, no significant differences were seen in PRRSV qPCR quantification following PMA treatment. Ultracentrifugation pre-treatment (with/without PMA or EMA treatment) has no effect on hgDNA and PRRSV quantification. Negative results were obtained by PVDA and HTS with untreated samples or samples treated only by ultracentrifugation. PRRSV was detectable by PVDA in EMA and PMA treated samples but PVDA best results were obtained following PMA treatment. HTS sensitivity was also improved by a treatment with EMA or PMA, but the number of reads was significantly higher in PMA treated samples. These results support the use of PMA as a treatment to increase sensitivity of PVDA and HTS.

083P

Development of monoclonal antibodies and other reagents for detection of porcine deltacoronavirus (PDCoV)

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Coronaviruses are enveloped, positive sense RNA viruses divided among four genera, including the recently described genus Deltacoronavirus. A novel porcine deltacoronavirus (PDCoV) was reported in China in 2012. In February 2014, the Ohio Department of Agriculture announced the identification of PDCoV in the U.S. Since then, PDCoV has been identified in a number of U.S. states and linked with apparent clinical disease including acute diarrhea and vomiting in the absence of other identifiable pathogens. PDCoV is currently diagnosed by real time PCR and clinical symptoms along with elimination of other viral pathogens known to cause similar disease. Since minimal specific antibody-based reagents are

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available to assist in diagnosis of PDCoV, the purpose of this study was to develop readily available reagents for detection of PDCoV antigen in diagnostic tests, such as virus isolation, immunohistochemistry and fluorescent antibody techniques.

The full-length nucleoprotein (NP) of PDCoV was cloned and expressed in *E. coli* as a 41 kDa polyhistidine fusion protein. This protein was purified by Nickel-NTA affinity column chromatography and is recognized in Western blotting and ELISA by convalescent serum from infected pigs. Both denatured and refolded versions of this protein were used to immunize rabbits for hyperimmune serum and mice for monoclonal antibody production. Rabbit hyperimmune sera specifically recognize the NP and can be used in indirect fluorescent antibody staining at dilutions of 1:1000 to 1:5000. To date, multiple hybridoma clones producing monoclonal antibodies against the PDCoV NP have been isolated and are currently being fully characterized.

The monoclonal antibodies, hyperimmune serum and related reagents produced in this project should prove of substantial value in the detection of PDCoV antigen following virus isolation attempts as well as in a variety of diagnostic methods such as immunohistochemistry, antigen capture assays and fluorescent antibody technologies. They are currently being utilized in PDCoV fluorescent focus neutralization (FFN) assays for assessment of neutralizing antibodies produced following infection.

084P

Evaluation of oral fluids as diagnostic specimens detecting porcine epidemic diarrhea virus (PEDV) shedding in experimentally infected weaned pigs

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Porcine epidemic diarrhea virus (PEDV) is an alphacoronavirus that causes severe diarrhea and weight loss in swine. This study investigated whether oral fluids and fecal swabs enable comparable sensitivities for detection of PEDV shedding in a herd of experimentally infected pigs. Ninety-six 3-week-old weaned pigs were randomly housed in 2 control groups (n=40) and 4 challenged groups (n=56). Challenged pigs were inoculated with 1 ml of  $1 \times 10^3$  plaque-forming units (PFU)/ml of PEDV isolate (US/Iowa/18984/2013) via gastric gavage. Control pigs received a sham inoculation of volume-matched virus-free cell culture media. Oral fluids were collected from the six groups (i.e. pens) using a rope collection method and fecal swabs collected from individual animals daily for the first seven days post-inoculation (dpi) and then twice a week through 35 dpi. Viral RNA was extracted, and RT-qPCR targeting the nucleocapsid gene of the PEDV was run alongside viral standards with known infectivity titers for quantification. Each cycle threshold value was then converted to viral titer (PFU equivalent/ml) estimated from a standard curve generated from the viral standards of known titers. Average viral load per dpi from group oral fluids and individual fecal swabs was calculated. The highest average viral load was detected at 3 dpi for both oral fluids and fecal swabs, with PFU/ml equivalents of  $6.99 \times 10^3$  and  $4.62 \times 10^4$ , respectively. Average viral loads for both specimen types gradually declined by 10 dpi, followed by a greater decrease by 35 dpi, when average PFU/mL equivalents for oral fluids and fecal swabs were 2 and 3, respectively. Viral RNA was detected in three of the four groups of oral fluids at 35 dpi while only one fecal swab tested positive. Oral fluids and fecal swabs from control animals consistently tested negative for PEDV RNA through the duration of the study. This study demonstrated oral fluids to be a valid specimen for population surveillance comparable in sensitivity to individual fecal swabs for detection of PEDV in pig herds via real-time RT-PCR.

085P

Quantification of uv-exposed porcine epidemic diarrhea virus using real-time rt-pcr

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**Purpose:** In order to prevent economic losses from PED, many swine producers use UV light as a sterilizer for the workers, equipment, surface of farm units etc. However, it is very little known about its actual degree of UV-induced RNA damage and effectiveness. The aim of the experiment was to measure levels of UV-induced RNA damage by utilizing the property that damaged RNA of PEDV can inhibit PCR.

**Methods:** The PED virus strain, P-5v, was used to assess the analytical performance of the reverse transcriptase PCR (RT-PCR) and real time-PCR (ReTi-PCR) protocols. This virus was treated by UV ramp (Enputech, Korea) with wavelength output at 254nm,  $150 \mu\text{W} \cdot \text{sec}/\text{cm}^2$ , for 0.5, 1, 2, 4, 8 and 16 min, respectively. RT-PCR amplified a 90bp of PEDV membrane protein (m) gene (337-426) region (Table. 1) and the amplicons were diluted  $10^{-5}$ -fold and analyzed by using SYBR<sup>®</sup> Green 1 Method for ReTi-PCR. To evaluate effects of UV induced RNA damage, Regression normalization (a linear trendline correction) was used. Table 1. Oligonucleotide primer.

**Results:** The inactivation ratio of PEDV was relatively quantified results from ReTi-PCR. The Ct value of serially diluted positive control sample showed the linear correlation ( $R^2=0.999$ ) (Fig. 1).

Fig 1. Linear trendline correction of standard curve of serially diluted control samples. Y axis is Ct value and X axis is  $-\log(\text{dilution ratio})-3$ . The inactivation of PEDV by UV light was dose dependent (Table. 2).

Table 2. The result of Ct value and inactivation ratio by exposure time using ReTi-PCR. In the setup of the experiment, 1D, 2D, and 3D (D=1log) inactivation were found 5.97, 46.33, and  $359.47 \text{ mJ}/\text{cm}^2$ , respectively.

**Conclusions:** UV is cost effective and environment friendly for disinfection of PEDV in pig farm units. It is thought that these results can be a useful data for sterilizing of PEDV.

086P

The induction of apoptosis in infected monocytes by cytopathic bovine viral diarrhea virus

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**Purpose:** Bovine viral diarrhea virus (BVDV) is an economically important viral pathogen in the livestock industry worldwide. BVDV exists in two biotypes, cytopathic (cp) and noncytopathic (ncp), based on their effect on cultured cells. Cp BVDV strains have been known to induce apoptosis, however the molecular processes controlling and executing apoptosis in vitro are not well understood.

**Methods:** Blood samples were collected, bovine PBMC were isolated using Histopaque gradients, and resuspended in RPMI 1640 medium. To separate monocytes, non-adherent cells were removed, and adherent cells were washed with sterile PBS, detached from the plates, and pelleted by centrifugation at 650 g for 10 min. For virus infection,  $5 \times 10^6$  monocytes were added to a 6 well plate and infected with cp and ncp BVDV biotypes at the same MOI of 0.002 for 48 h. After 48 h, ncp BVDV, cp BVDV, and mock-infected cells were harvested. Annexin V-FITC



## Viral Pathogenesis Posters

(086P continued)

staining, TUNEL assay, and western blots were performed. TNF $\alpha$  concentrations from cell culture supernatants were measured by ELISA. Results: Annexin V staining showed that an increased expression of PS in cp BVDV-infected monocytes. Typical apoptotic morphology was observed in cp BVDV-infected monocytes, whereas ncp BVDV-infected and mock-infected monocytes exhibited low proportions of apoptotic cells. The caspase-3 enzymatic activity of cp BVDV-infected monocytes was relatively higher than that of ncp BVDV-infected cells. The expression of the active 35-kDa caspase-3 was significantly increased in cp BVDV-infected monocytes. Mx1 protein expression was induced only in cp BVDV-infected monocytes. TNF $\alpha$  secretion was elevated in cell culture supernatants of cp BVDV-infected monocytes compared to ncp BVDV-infected ones.

Conclusions: Our results showed that cp BVDV induce cell death, virus-induced IFN expression (Mx1) and the increased production of TNF $\alpha$ . These findings may help to further understanding of the pathogenesis of MD, highlighting cp BVDV-specific cellular responses.

Acknowledgments: This work was supported by grant NRF 2012R1A1A3011238.

087P

Assessment of ultraviolet irradiation-induced RNA damage on classical swine fever virus by realtime RT-PCR

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Purpose: Classical Swine Fever (CSF) as RNA virus is a highly contagious disease of pigs. It is classified as List A-grade disease determined by Office International des Epizooties (OIE). Because of its extremely high mortality with severe symptoms, UV light is used by many pork producers. However, it is very little known about actual effects of UV-induced RNA damage. The aim of the experiment was to measure levels of UV-induced RNA damage by utilizing the property that damaged RNA of CSFV can inhibit PCR. Methods: The CSF virus strain, LOM strain, was used to assess the analytical performance of the reverse transcriptase PCR (RT-PCR) and real time-PCR (ReTi-PCR) protocols. This virus was treated by UV ramp (Enputech, Korea) with wavelength output at 254nm, 150 $\mu$ W $\cdot$ sec/Cm $^2$ , for 1, 2 and 4 min, respectively. RT-PCR amplified a 99bp (7824-7922) region (Table. 1) and the amplicons were diluted 10 $^{-5}$ -fold and analyzed by using SYBR $^{\circledR}$  Green 1 Method for ReTi-PCR.

Statistical evaluation was performed by Excel (Microsoft, USA), Using regression normalization (a linear trend line correction).

Table 1. Oligonucleotide primer

Results: The inactivation ratio of CSFV was relatively quantified results from ReTi-PCR. The Ct value of serially diluted positive control sample showed the linear correlation ( $R^2=0.999$ ) (Fig. 1).

Fig 1. Standard curve of serially diluted control samples Y axis is Ct value and X axis is Log(dilution ratio)+8.

The inactivation of CSFV by UV light was dose dependent (Table. 2).

Table 2. The result of Ct value and inactivation ratio by exposure time using ReTi-PCR

In the setup of the experiment, 1D, 2D, and 3D inactivation were found 23.89894, 101.3937 and 430.1734 mJ/cm $^2$ , respectively.

Conclusions: UV is cost effective and environment friendly for disinfection of CSFV in pig farm units. It is thought that these results can be a useful data for sterilizing of CSFV.

088P

Infection of post-weaning gnotobiotic pigs with genogroup II human norovirus.

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Purpose: Viral gastroenteritis caused by human norovirus (HuNoV) infection is serious foodborne disease worldwide. But the pathogenesis of HuNoV is poorly understood because cell-culture system and proper animal model are not available. However, gnotobiotic pigs can be an alternative animal model to study the pathological and immunological responses to HuNoV infection. Since gnotobiotic pigs have no exposure to viral pathogens and have A and H histo-blood group antigen of humans, they can be currently the most optimal animal model for study of HuNoV pathogenesis. In this study, we evaluated clinical signs, viral shedding, and replication of virus in small intestine after infection of post-weaning gnotobiotic pigs with HuNoV.

Methods: Eight post-weaning gnotobiotic pigs were used in this study. Three groups of pigs, 2 pigs in each group, were orally inoculated with genogroup II genotype 4 (GI.4) HuNoV having 1.78 x 10 $^5$ , 1.78 x 10 $^6$ , and 1.78 x 10 $^7$  RNA copies, respectively. Two pigs were used as negative control group. Blood and rectal swab samples were collected for 3 days and gross lesions were examined after euthanization.

Results: Viral shedding was detected in feces for up to 3 days in the two pigs infected with 1.78 x 10 $^6$  viral copies. The viral titers of the fecal samples reached to 1.7 x 10 $^6$  copies/ml. Negative sense RNA, direct evidence of NoV replication, was detected in the jejunum and ileum of infected pigs. Viremia was also detected in one pig.

Conclusions: These data indicate that post-weaning gnotobiotic pigs can be useful animal model to study pathogenesis of HuNoV.

089P

Detection of hepatitis E virus in rabbits in Korea.

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Purpose: Hepatitis E virus (HEV) is a significant human pathogen worldwide. Four established and two putative genotypes have been reported: genotype 1 and 2 only infect humans, whereas genotype 3 and 4 are recognized as zoonotic agents. Along with this, there is growing interests on rabbits which are reservoirs for HEVs that can cause infection in humans. Therefore, it is necessary to isolate rabbit HEV and to study pathogenicity of it. Rabbit HEVs have been isolated from several countries, but there is no report of detection of rabbit HEV in Korea. In this study, we tried to detect rabbit HEV and to analyze genetic characteristics of it.

Methods: We extracted viral RNA from rabbit feces and detected HEV sequence by nested PCR using modified JM primer sets. DNA sequence of the PCR product was compared with other HEV sequences.

Results: The analysis demonstrated that the DNA sequence obtained in this study was closely related with rabbit HEV. These findings are further confirmed by a phylogenetic tree analysis.

Conclusions: In conclusion, we report for the first time isolation of rabbit HEV in South Korea. We expect this rabbit HEV will be used for development of vaccine and for understanding of viral pathogenesis.

# **ORAL ABSTRACTS**

## Bacterial Pathogenesis

001

Identification of a Type II Toxin-antitoxin System in *Campylobacter jejuni*

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Toxin-antitoxin (TA) systems are prevalent in various bacterial organisms. These systems are pairs of genes that encode a stable toxin and an unstable antitoxin and their primary function in the plasmid is to maintain the plasmid by eliminating plasmid-free daughter cells through a post segregation killing mechanism. Our recent study first demonstrated that the existence of the type I and type II TA systems in pVir plasmid of *C. jejuni*. In this study, we report the identification and characterization of the type II toxin-antitoxin system in the pVir plasmid of *C. jejuni* IA3902, a highly abortifacient isolate from sheep. Cloning and expression of the toxin gene (CJSA\_pVir0046) of this TA system in *Escherichia coli* resulted in expression-dependent growth inhibition. This growth inhibitory effect was eliminated by coexpression of the antitoxin gene (CJSA\_pVir0045) along with the toxin gene. The purified toxin protein did not have endoribonuclease activity by itself, but it could inhibit protein synthesis *in vitro* assay. These results indicated that CJSA\_pVir0046 probably functions like other members of RelE family, which have ribosome-dependent endogenous ribonuclease activity. In *C. jejuni* 3902, inactivation of CJSA\_pVir0046 resulted in rapid loss of the pVir plasmid in progeny cells, while the wild-type strain stably maintained the plasmid despite prolonged passage in culture media, indicating the importance of the TA system for the maintenance of pVir in *C. jejuni* IA3902. Together, these results establish that CJSA\_pVir0046 and CJSA\_pVir0045 encode a functional TA system in *C. jejuni*.

002

A single nucleotide change in *mutY* leads to the increased emergence of fluoroquinolones resistant mutants in *Campylobacter jejuni*

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*Campylobacter jejuni* is a major foodborne pathogen. Its resistance to clinically important antibiotics (macrolides and fluoroquinolones) is a major public concern and is primarily mediated by mutations in the antibiotic target genes. But the genetic factors that affect spontaneous mutation frequencies are still poorly understood in *Campylobacter*. In this study, a highly mutator *C. jejuni* strain CMT, which has 50~100 fold higher spontaneous ciprofloxacin resistance (Cip<sup>R</sup>) frequency than the *C. jejuni* reference strain 11168 strain, was submitted for whole genome sequencing in order to find potential mutator factors in CMT isolate.

Illumina platform was utilized to sequence the whole genome of *C. jejuni* strain CMT. The assembled CMT whole genome sequence was mapped to *C. jejuni* 11168 isolate. To examine the role of potential target gene *cj1620c*, which was predicted to encode the homolog of DNA repair protein MutY in *C. jejuni*, an intact copy of this gene from 11168 strain was cloned into the 16S region of CMT strain. Further analysis of a nucleotide change in *mutY* gene of CMT strain, *in vivo* site directed mutagenesis was utilized to replace the nucleotide of *mutY* in CMT with that in 11168 strain. Subsequently, all the *C. jejuni* constructs were tested for the spontaneous Cip<sup>R</sup> frequency.

Sequencing result showed *C. jejuni* CMT and 11168 share almost identical genome sequence with 11168. However, a total of 24 nucleotide changes or frame shift were observed in CMT strain. Among these mutations, there is a C to A transversion in the putative *mutY* gene in CMT strain, which leads to 199 G-W amino acid change in its encoding sequence. Coexistence of *mutY* copies from both 11168 and CMT strains only slightly reduced the spontaneous Cip<sup>R</sup> frequency. However, after the reverse mutation of 199 W-G in the MutY, the spontaneous Cip<sup>R</sup> frequency was reduced by ~100 fold in CMT strain. In the meantime, 199 G-W mutation of MutY increase the spontaneous Cip<sup>R</sup> frequency in 11168 strain as expected.

The C to A transversion strongly modulates the function of *mutY* gene, which in consequence, promotes the emergence of fluoroquinolones resistant mutants in *Campylobacter*.

003

Transcriptomic responses of a *Campylobacter jejuni* strain associated with sheep abortion to sheep whole blood and identification of a novel abortion-inducing factor

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A highly pathogenic *Campylobacter jejuni* clone (SA for sheep abortion) has recently emerged as the major cause of *Campylobacter*-associated sheep abortion in the U.S. and is transmitted to humans via raw milk and other unknown routes, causing gastroenteritis. Following the natural infection route of oral ingestion, *C. jejuni* must translocate from the intestinal lumen to the bloodstream, in order to reach fetoplacental tissue and induce abortion. Thus, survival in blood is essential for systemic spread, but how *C. jejuni* adapt in blood remains poorly understood. In this study, we performed a time-course transcriptome analysis of *C. jejuni* survival using an ex vivo model of sheep whole blood infection. We observed that *C. jejuni* SA altered the expression of ~36% (600/1666) of its ORFs. The major dynamic changes in expression were associated with genes involved in transcription and translation, amino acid transport and metabolism, inorganic ion transport and metabolism, chaperones, cell wall/membrane biogenesis, energy production and conversion, signal transduction mechanisms and cell motility. Through mutagenesis studies of a subset of up-regulated genes, five genes were identified to be important for survival in sheep blood. Of these, *cjsa\_0039* which encodes a GTP-binding protein, was demonstrated to play a key role in abortion induction using guinea pig model. These results increase the knowledge of how *C. jejuni* adapts in blood and provide new insight into *C. jejuni* pathogenesis of abortion induction.

004

Environmental persistence and biofilm formation of hypermucoid and non-hypermucoid *Klebsiella pneumoniae*

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In recent years, an emergent *Klebsiella pneumoniae* hypermucoviscosity (HMV) phenotype has been associated with increased invasiveness and pathogenicity in primates. The HMV phenotype is characterized by different capsular serotypes, associated with several virulence genes including the *rmpA* (regulator of mucoid phenotype) and *magA* (mucoviscosity-associated) gene. In African green monkeys (AGM)

## Bacterial Pathogenesis

(004 continued)

(*Chlorocebus aethiops sabaeus*) serotypes K1 and K5 have been implicated in fatal multisystemic abscesses. In order to better understand the epizootiology of this pathogen, environmental samples were recovered from different sites in a non-human primate research facility where *K. pneumoniae* have been isolated from clinically affected animals during the past 5 years. The same sites were sampled four times during 2013, and total DNA was extracted from swab suspensions and served as template for the detection of genes previously associated with invasive diseases and hypermucoviscosity by real time PCR. Non-HMV *K. pneumoniae* was detected in 4 different sites, whereas the *magA* gene (indicative of serotype K1) was only detected in one of the sites. Additionally, the capacity of biofilm production of *K. pneumoniae* isolates presenting the HMV was compared to non-HMV isolates at three different temperatures (25, 30 and 37° C). The results indicate that non-HMV isolates displayed significantly greater capacity to form biofilms than the corresponding isolates presenting the HMV phenotype at the three different evaluated temperatures. Temperature also appears to play a role in the formation of biofilms by *K. pneumoniae* presenting the HMV phenotype, where larger biofilms were formed at 25° C than at 37° C. Knowledge regarding local environmental sources of *K. pneumoniae* and the possible role of wildlife in the maintenance of this agent in the area is necessary to develop effective recommendations for the prevention and management of this disease in captive AGM populations.

005

Characterization of Glucosamine-6-Phosphate Synthase (GlmS) mutant of *Salmonella*: Effect on Biology and Pathogenesis of the Organism  
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*Salmonella enterica* serovar Enteritidis (SE) is one of the most common causes of food-borne illnesses in the United States and is a significant cause of morbidity and mortality. The prevalence of *Salmonella* in meat and poultry products highlights the importance of this organism in human health. In the United States, the number of prevalent cases of salmonellosis has been estimated to be 1.39 million per year with 415 deaths. Identification and characterization of factors involved in *Salmonella* pathogenesis and metabolism would help develop effective controls and treatments. Using random mutation library, we identified *glmS* as defective in growth and binding of *Salmonella* to intestinal epithelial cells. *GlmS* encodes an important aminotransferase, glucosamine -6-phosphate synthase, which converts fructose-6-phosphate and glutamine to glucosamine-6-phosphate. This enzyme participates in the hexosamine pathway, producing a precursor to the formation of N-acetylglucosamine (GlcNAc). GlcNAc is an important amino sugar in Gram-negative bacteria, as it is a vital component of the peptidoglycan and lipopolysaccharide layers, important for bacterial cell wall integrity. We hypothesize that *GlmS* plays a vital role in maintaining *Salmonella* outer membrane integrity and affects the ability of the organism to survive inside host cells. To test this, we created a  $\Delta$ *glmS* deletion mutant of SE and subsequently investigated the effects of the deletion on growth of the organism, compared to wild-type (WT). We further examined the integrity of the cell wall using detergents and hydrophobic antibiotics along with cellular morphological evaluation of the bacteria. Data from these experiments indicate that  $\Delta$ *glmS* was defective in growth compared to the WT and the complemented strains. Supplementation of media with D-glucosamine was able to restore WT growth characteristics in the mutant. There was a significant compromise to the bacterial cell wall as indicated by sensitivity to detergents and hydrophobic antibiotics. These findings indicate *glmS* encodes important components affecting the growth and integrity of the bacterial cell wall.

006

Human specific virulence factors absent in LA-MRSA ST5 strains isolated from pigs, swine facilities, and humans with swine contact.

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**Purpose:** Since its first detection associated with the swine industry, livestock associated methicillin-resistant *Staphylococcus aureus* (LA-MRSA) has drawn concern from the public health community because livestock may be the largest reservoir of MRSA outside of hospital settings. In contrast to Europe, where the predominant sequence type (ST) of LA-MRSA associated with swine is ST398 and Asia where ST9 isolates predominate, US isolates appear to be a mixed population with ST5 being commonly isolated as well as ST398. The presence of ST5 isolates in swine is a particular cause for concern because ST5, unlike ST398 and ST9, is one of the most successful and globally disseminated lineages of MRSA associated with human disease. This success is believed to be due to ST5 strains being more prone to acquiring virulence or host-adapted genes by bacteriophages. The general absence of known virulence factors in ST398 MRSA isolates from swine has been established and the lack of reports of human disease caused by ST5 MRSA in people occupationally exposed to swine suggests that ST5 strains in animals may also lack genes necessary for causing human disease.

**Methods:** ST5 isolates were obtained from swine, swine facilities, individuals with transient swine contact, and from individuals with long-term exposure to swine. These isolates were evaluated by PCR for the presence of the human-specific virulence factor staphylokinase and genes carried by  $\beta$ -hemolysin-converting bacteriophages.

**Results:** All LA-MRSA ST5 isolates tested were positive via PCR for an intact  $\beta$ -hemolysin gene. This demonstrates that the  $\beta$ -hemolysin-converting bacteriophage is not present in any of the isolates tested. All isolates tested were also negative via PCR for the staphylokinase gene.

**Conclusions:** The absence of the  $\beta$ -hemolysin-converting bacteriophage and the staphylokinase gene is consistent with the hypothesis that *S. aureus* strains adapted to animals generally lack the capacity to cause significant disease in immunocompetent humans.

007

Global control of virulence and survival of *Mycobacterium avium* subsp. *paratuberculosis*.

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Johne's disease (JD) or paratuberculosis in animals (infection with *M. avium* ss. *paratuberculosis*, *M. ap*) is a contagious, chronic, and potentially fatal disease affecting ruminants. The largest economic impact is felt in dairy herds, where infected cattle suffer from chronic diarrhea, weight loss, low milk yield and low (but persistent) mortality. To improve our understanding of *M. ap* pathogenesis, we examined key regulators of gene expression, mainly sigma factor H and L (SigH and SigL), among the 19 sigma factors encoded in *M. ap* genome. Earlier analysis in our group indicated the differential regulations of the sigH and sigL genes during macrophage infection. SigH is induced in response to heat, oxidative stress, and it was shown to be important during persistent infection in *M. tuberculosis*. In this study, a specialized transduction protocol was

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applied to delete sigH and sigL genes from the genome of *M. ap* K10. After construction, the resistance of the  $\Delta$ sigH and  $\Delta$ sigL mutants was evaluated against various stressors. The  $\Delta$ sigH mutant showed increased sensitivity to a sustained level of thiol-specific oxidative stress and heat treatment while  $\Delta$ sigL showed increased sensitivity to diamide oxidative stress and acidified bovine bile. Large-scale RNA sequence analysis revealed that sigH modulates a large number of genes, especially following diamide stress. Genes involved in heat-shock response, oxidative stress and virulence were among the induced genes in the sigH regulon with a putative consensus sequence for SigH binding was recognized in the upstream of at least 30 genes, suggesting direct regulation with SigH. On the other hand, proteomic analysis of the sigL mutant indicated the differential regulation of 113 proteins. Finally, both mutants were significantly attenuated in the murine model of infection compared to its parental strain suggesting a pivotal role for the examined sigma factors in *M. ap* virulence. Taken together, our results suggest that sigH and sigL are required for *M. ap* pathogenesis and both could provide promising candidates for the development of a potent vaccine desperately needed against Johne's disease.

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008

Overexpression of catalytically inactive dimethyl adenosine transferase (KsgA) unveils contribution of KsgA to *Salmonella* Enteritidis physiology and virulence

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**Purpose:** *Salmonella* Enteritidis (SE) is a major cause of food-borne gastroenteritis in humans, its primary source being contaminated poultry products. Dimethyl adenosine transferase (KsgA) performs bacterial ribosomal maturation, DNA mismatch repair, and its synthesis is responsive to antibiotics and cold temperature. KsgA mutation in SE results in impaired invasiveness in human intestinal epithelial cells, increased sensitivity to high osmolarity, oxidative stress and antibiotics such as chloramphenicol. Interestingly, overexpression of a catalytically inactive form of KsgA (KsgA::E66A) was reported to result in severe growth defects in *E. coli*. The aim of this study was to determine the effects of overexpression of KsgA::E66A as a tool to unveil the contribution of KsgA to SE physiology and virulence.

**Methods:** Catalytically inactive KsgA (KsgA::E66A) was overexpressed in wild-type (wt) and KsgA deficient mutant (*ksgA::Tn5*) of SE and tested for i) Growth at avian body temperature (42°C) in nutrient rich media (LB), ii) motility in Muller-Hinton agar, iii) invasion of avian hepatocellular carcinoma cells (LMH), and iv) colony morphology by light microscopy and transmission electron microscopy (TEM).

**Results and Conclusions:** Overexpression of KsgA::E66A, in wt and *ksgA::Tn5* SE strains resulted in i) mild growth defect during late log phase at avian body temperature; ii) reduced invasiveness of chicken

epithelial cells and iii) wrinkled colony phenotype on LB agar. These phenotypes were more pronounced in the *ksgA::Tn5* as compared with the wt. No differences were observed in motility of these strains. However, TEM of the wt overexpressing KsgA::E66A revealed changes in outer membrane (OM) structure and apparently reduced number of ribosomal figures. TEM studies on *ksgA::Tn5* are currently ongoing and it is expected that these effects will be more pronounced. The results suggest that overexpression of KsgA::E66A significantly alters SE physiology and may result in virulence attenuation, making it a potential candidate for drug and/or vaccine development. Ongoing research aims to study possible applications of KsgA::E66A in prevention and control of SE in the avian host.

009

Towards an understanding of *Salmonella* persistence in pigs.

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*Salmonella enterica* is one of the most common causes of food borne diarrheal disease. *S. enterica* is believed to enter the food chain is by first establishing long-term persistent infections of pigs. Persistent infections are established early in life and once established can persist for the life of the animal. Persistently infected pigs intermittently shed *S. enterica* in feces in low numbers. Even though pigs show no signs of infection and may only shed the organism in feces sporadically, stresses including transport and feed withdrawal promote the resumption of fecal shedding just prior to. Thus, swine can act as a reservoir for the spread of *S. enterica*. *Salmonella enterica* serovar Typhimurium strain 798 is known to undergo phenotypic phase variation. One of the phenotypes expresses virulence traits while the other phenotype does not. Phenotypic phase variation correlates with the ability of this strain to cause persistent infections of swine. The over expression of the regulatory gene *LrhA* causes the rate of phase variation to increase. The rate of phase variation from non-adhesive to adhesive phenotype was approximately  $10^{-4}$  per cell per generation while phase variation from the adhesive to the non-adhesive phenotype was approximately  $10^{-6}$  per cell per generation. Two highly virulent *S. Typhimurium* strains were shown to undergo phase variation. However, the rate of phase variation from the non-adhesive to adhesive phenotype shift was 37-fold higher. Differential gene expression was measured and eighty-three genes were more highly expressed by 798 cells in the adhesive phenotype compared to the non-adhesive cells. Most of the up-regulated genes were in virulence genes including all of the genes encoded in the *Salmonella* pathogenicity island 1. When compared to the virulent strain SL1344, expression of the virulence genes was approximately equal to those up-regulated in the adhesive phenotype of strain 798. Infection of pigs with *S. enterica* (naturally or experimentally) was shown to be correlated with significant changes in the composition of the gut microbiome including decreases in *Prevotella*, *Lactobacillus*, and *Campylobacter* and increases in *Barnesiella* and *Pseudobutyrvibrio*.

010

Brucellosis in animal reservoirs in the United States: challenges and opportunities

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**Purpose:** *Brucella* spp are bacterial pathogens associated with reproductive losses and zoonotic disease. Because of infections in humans, brucellosis regulatory programs were first initiated in the 1930's. Although essentially eradicated from domestic livestock, brucellosis (*Brucella abortus*, *B. suis*) continues to be maintained in wildlife reservoirs with frequent spillover back to domestic livestock. The infection of bison and elk in the area in and surrounding Yellowstone National Park is controversial, not only because of differences in opinion regarding disease risks, but also because the issues are complex and involve numerous state and federal agencies with conflicting missions. The expansion of the range of feral swine in the U.S. and the associated issues with swine brucellosis are causing regulatory and zoonotic issues in numerous states. Although vaccination combined with test and removal has been effective in disease mitigation in domestic livestock, implementation of similar approaches in wildlife is more problematic. Susceptibility to infection and immunologic responses to vaccination or infection appear to differ across host

## Bacterial Pathogenesis

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species. Resolution of brucellosis in wildlife reservoirs in the U.S. will require development of new and innovative tools and approaches, and likely be as expensive as the cost for the regulatory program in cattle.

011

Bovine tuberculosis eradication: Update and emerging issues

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*Mycobacterium bovis* is the causative agent of tuberculosis in animals. Infection in humans can mimic disease caused by *M. tuberculosis*. Public health concerns over the transmission of *M. bovis* from cattle to humans prompted the United States to initiate a bovine tuberculosis eradication campaign in 1917. Great progress has been made in eliminating this zoonotic pathogen using tuberculin skin testing and a “test and slaughter” approach; however, today obstacles to eradication have arisen that pose serious challenges to disease eradication. These obstacles include, importation of infected cattle from Mexico, a need for improved diagnostic methods that allow testing and removal of infected animals, rather than the extreme of whole herd depopulation, and the presence of *M. bovis* in wildlife, specifically white-tailed deer. In northeast Michigan, free-ranging deer represent a reservoir of *M. bovis*, with regular interspecies transmission. Efforts to decrease the number of deer through increased hunting have decreased the apparent prevalence, but further decreases in deer population are unlikely. Recent research has resulted in novel diagnostic tests that have been licensed and approved for use in both cattle and deer. Vaccination of deer with *Mycobacterium bovis* bacillus Calmette-Guerin (BCG) has been shown to reduce disease severity and may be useful in decreasing deer-to-deer and deer-to-cattle transmission of *M. bovis*. As both oral and parenteral routes of administration provide equal protection, oral baits for delivery to free-ranging white-tailed deer may be possible.

012

Arbovirus research and epizootic hemorrhagic disease

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The Arthropod-Borne Animal Diseases Research Unit (ABADRU) is one of five units at the Center for Grain and Animal Health Research (CGAHR). The ABADRU has four 5-year project plans under two ARS National Research Programs; Animal Health NP103 and Veterinary, Medical, and Urban Entomology NP 104. These plans include research on bluetongue virus (BTV; exotic and domestic), epizootic hemorrhagic disease (EHD), vesicular stomatitis virus (VSV), and Rift Valley fever virus (RVFV). Transmission of arboviruses and disease events are largely driven by complex and often unknown factors related to vector biology and ecology, as well as host, virus and environmental factors. Epizootic hemorrhagic disease virus (EHDV) is an arthropod-transmitted virus closely related to bluetongue virus in the genus *Orbivirus* of the family *Reoviridae*. Three of the seven known EHDV serotypes circulate in the USA, EHDV-1, -2, and most recently EHDV-6. These viruses infect a variety of wild and domestic ruminant hosts, although the susceptibility to clinical disease varies greatly among host species. Among wild ruminants, none are more susceptible to severe disease than white-tailed deer (WTD; *Odocoileus virginianus*) and EHD is one of the most significant diseases of this species. However, infection of domestic cattle with EHDV is also common and while frequently subclinical, reports of disease associated with EHDV infection in cattle have increased in recent years. While *Culicoides sonorensis* is the only confirmed EHDV vector in North America, additional *Culicoides* spp. likely play a role in EHD transmission and numerous suspected vectors exist. Since the initial detection and characterization of EHDV in New Jersey WTD the 1950s, these viruses have circulated in endemic and epidemic patterns with occasional incursions to more northern latitudes. In recent years, changes in the pattern of *Orbivirus* infection and disease have forced the scientific community to revisit some fundamental areas of research and have exposed our incomplete understanding of EHD epidemiology, especially concerning overwintering mechanisms and competent vector populations.

013

Has changing animal husbandry practices changed the ecology of influenza?

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Since the middle-ages there have been sporadic reports of fast moving epidemics in people that resembled what is now known as influenza. The onset was typically associated with the movement of large groups of people, usually an invading army and its fleeing victims. Forensic studies have provided empirical evidence that similar 19<sup>th</sup> century human epidemics were related to influenza A virus infection lending support to the assumption that many of the ancient epidemics were influenza pandemics. Epidemics in domesticated animals and poultry were frequently reported prior to or following the onset of human disease leading contemporary observers to presume a causal relationship among involved species. Clinical signs resembling what is now known as equine influenza were reported in horses during these outbreaks and they seemed to be the most affected animal. Presumably, their high concentration in military campaigns and close association with humans contributed to disease in both species. Technological advances have revealed the dynamic ecology of influenza A viruses, especially the relationship between swine and man which was not recognized until the onset of the 1918 Spanish Flu pandemic. Interestingly, coincident with the onset of a swine/human influenza interaction was the apparent loss of the equine/human linkage suggesting swine may have displaced horses as a mammalian reservoir for human influenza A viruses. Moreover, over the last 20 years there have been many novel influenza A subtypes found in pigs which may reflect more sensitive diagnostic methods, but may represent a changing role of swine as a mammalian reservoir of influenza A viruses for humans. The consequences of such events will be discussed along with possible actions to mitigate such events.

014

*Mannheimia haemolytica* biofilm formation on bovine respiratory epithelial cells

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*Mannheimia haemolytica* is the main bacterial agent associated with the bovine respiratory disease complex, which causes worldwide economic losses to the cattle industry. The *M. haemolytica* cells initially colonize the tonsillar crypts in the upper respiratory tract of cattle as a commensal, and subsequently descend into the lungs to cause disease. Many bacterial species colonize their host as a biofilm, thereby allowing the bacterial cells to persist amidst antimicrobial therapy and immune responses. We previously reported that *M. haemolytica* forms biofilms on an abiotic

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surface (plastic) in vitro. In this study we developed a model for studying *M. haemolytica* biofilm formation on bovine respiratory epithelial cells. Using fixed primary bovine bronchial epithelial cells, we observed *M. haemolytica* biofilm formation after a 48 hours incubation period. Monosaccharides have been used by other investigators to block cellular adhesion. We hypothesized that adding certain monosaccharides might reduce adhesion and biofilm formation by *M. haemolytica* on bovine epithelial cells. Biofilm formation was inhibited by adding the monosaccharides D (+) mannose, methyl  $\alpha$ -glucoside, D (+) galactose, or L (-) fucose to the growth medium. We then compared the resistance of *M. haemolytica* planktonic cells and biofilm cells formed on plastic to the antibiotics gentamicin, florfenicol, and tulathromycin. We found that the concentrations of gentamicin and florfenicol required to reduce the CFU of biofilm cells was greater than the concentrations needed to kill planktonic cells. Ongoing experiments are investigating the antibiotic resistance of *M. haemolytica* biofilm cells on bovine epithelial cells. If our results show that *M. haemolytica* biofilm cells are more resistant than planktonic cells to antibiotics, this characteristic could contribute to the ability of *M. haemolytica* to persist as a commensal in the upper respiratory tract of cattle.

015

Characterization of a novel isolate of *Leptospira* associated with the greater white-toothed shrew (*Crocidura russula*), an invasive mammalian species in Ireland.

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The greater white-toothed shrew (*Crocidura russula*) is an invasive species in Ireland that was first recorded in 2007. *C. russula* was discovered in Ireland from pellets of barn owls (*Tyto alba*) and kestrels (*Falco tinnunculus*) collected in 2007, with later confirmation by live-trapping in 2008. *C. russula* is distributed in Northern Africa and Western Europe, and was previously absent from the British Isles. Whilst invasive species can have dramatic and rapid impacts on faunal and floral communities, they may also be carriers of pathogens facilitating disease transmission in potentially naïve populations. The goal of this study was to determine if *C. russula* was a reservoir host for pathogenic species of *Leptospira*. Culture of kidneys from 18 trapped *C. russula* yielded 3 isolates of *Leptospira*, using EMJH medium supplemented with 0.2% Noble agar and 200ug/ml 5-Fluorouracil. None of the isolates were reactive with reference antisera against 17 serovars representing 15 serogroups of *Leptospira*. Antisera generated against each of the three isolates caused agglutination at 1:1000, 1:3000 and 1:10 respectively against *L. santarosai* serovar Shermani suggesting that these isolates belong to serogroup Shermani. However, whilst Restriction Enzyme Analysis (REA) of DNA from isolate #1 and #3 was identical, and both were similar to isolate #2, all were significantly different to the REA profile of serovar Shermani. Experimental infection of Golden Syrian Hamsters with 10<sup>7</sup>, 10<sup>5</sup> and 10<sup>3</sup> of isolate #1 confirmed pathogenicity: kidneys of all hamsters were culture positive as were 5 out of 6 liver samples when hamsters were euthanized on days 5, 8 and 10 post-infection respectively. This study demonstrates that invasive mammalian species may act as bridge vectors of novel zoonotic pathogens such as *Leptospira*.

016

Complete genome sequencing and analysis of the red blood cell pathogen of llamas and alpacas, '*Candidatus Mycoplasma haemolamae*'.

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'*Candidatus Mycoplasma haemolamae*' (CMhl) can cause a chronic, long-lasting infection or a life-threatening, acute disease characterized by severe anemia in llamas and alpacas. Mechanisms by which CMhl evades the immune system and causes chronic infection, as well as triggers of acute disease, are poorly understood. Thus, the sequencing and analysis of CMhl genome was undertaken to gain new insights into its metabolism and pathogenesis. Bacterial organisms were harvested from the blood of a naturally infected, splenectomized alpaca at peak of bacteremia. Genomic DNA was extracted using Quick-gDNA midiprep kit (Zymo Research Corp.) and genome sequencing was performed using Roche 454 GS-FLX to sequence a genomic library according to a rapid library prep protocol. Reads were assembled using gs-Assembler 2.3 (Life Technologies), resulting in 7 scaffolds (1,118 - 731,011 bp). For gap closure, the same sample was used to obtain Illumina reads from a paired-end library. Reads were assembled using ABYSS-PE v1.2.7 (Illumina) and predicted scaffolds were used to manually close the gaps. Genomic annotation was done using NCBI pipeline. The CMhl genome is composed of a small, circular chromosome of 756,845 bp with 39.3% of GC. A total of 925 protein-coding sequences (CDSs) were identified, and the rRNA genes were found in single copies and unlinked. Only 280 (30.3%) CDSs have known functions, while 645 (69.7%) are hypothetical proteins. The predicted metabolic pathway is concise and similar to other members of the *Mycoplasma suis* group. It shows evidence of adaptation to the blood environment and of energy acquisition through sugars fermentation and ATP-synthase. Toxins homologs were not found. As proposed for other hemoplasmas, CMhl may cause disease by scavenging and competing for the host' nutrients, leading to decreased erythrocyte lifespan in acute disease. Also, 49.1% of the CDSs are organized in paralogous gene families. Some of these families may be related to antigenic variation and bacterial persistence within the host. In conclusion, genome analysis shows that CMhl is dependent on host cell metabolism for its survival and this characteristic is likely to be linked to its pathogenicity.

017

Insulated isothermal PCR assay for the detection of *Taylorella equigenitalis*

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Contagious equine metritis (CEM) is a sexually transmitted disease of horses caused by *Taylorella equigenitalis* (Teq). Early detection and treatment of carrier mares and stallions are important in prevention and control of CEM. In this study, an insulated isothermal PCR (iiPCR) assay targeting the gene encoding Hep\_Hag family protein was developed to facilitate point-of-need CEM diagnosis. The iiPCR was designed for use on POKKIT<sup>TM</sup> Nucleic Acid Analyzer, a field-deployable device capable of generating automatically interpreted results within one hour. LoD 95% of the Teq iiPCR per reaction was about 18 copies of linearized plasmid DNA which containing partial sequence of the target gene. In addition, detection of Teq DNA with the Teq iiPCR was not interfered by horse genomic DNA. Excellent specificity of the test was obtained using 16 strains of Teq and 8 strains of *T. asingenitalis* (a closely related bacterium), as well as *Leptospira* spp, *Streptococcus zooepidemicus*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, and *Escherichia coli*. Performance of Teq iiPCR assay was compared to that of a currently used real-time PCR assay (Wakeley et al., 2006). Limits of detecting Teq DNA were similar (10 copies of genomic DNA)

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between iiPCR and the reference real-time PCR. In the presence of 50,000 copies of *T. asinigenitalis* genomic DNA, the detection limits for iiPCR and the reference real-time PCR were 10 and >1000 copies of Teq genomic DNA, indicating that Teq iiPCR could tolerate more *T. asinigenitalis* genomic DNA than the reference assay. Test results with nucleic acids prepared from an uro-genital swab sample spiked with different concentrations of Teq showed that the detection end-points of the reference method and iiPCR were at  $10^3$  and  $10^6$  dilutions of Teq, respectively.

In conclusion, compared to the reference real-time PCR, Teq iiPCR assay had comparable sensitivity and could tolerate more interference from contaminating DNA in equine samples.

The established assay is a rapid, sensitive, and specific tool for easy point-of-need detection of Teq in uro-genital specimens from horses.

018

An internal control improves the detection of *Brucella canis* in diagnostic samples analyzed by a triplex real-time PCR assay

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*Brucella canis* is the primary causal agent of canine brucellosis that causes reproductive disorders. The disease is contagious and the organism persists longer in animals than other *Brucella* species. Although mortality is relatively low, morbidity is high. We have previously developed a duplex real-time PCR (qPCR) assay to detect all *Brucella* species, as well as *B. canis* specifically. The common molecular target was designed from the spacer region between the 16S and 23S rRNA genes that is present in all *Brucella* species. The *B. canis*-specific target was designed flanking a nearly 1 kb deletion that occurs only in *B. canis* strains. Our previous study indicated that this duplex qPCR detected approximately 50% more positives than the traditional culture method (3 PCR positives for every 2 culture positives). A total of 3,975 canine whole blood samples were submitted to Kansas State Veterinary Diagnostic Laboratory (KSVDL) during a 17-month period. Among these submissions, 220 were tested positive for *Brucella canis*, which is 5.5% of the total sample size. In comparing 24 h and 48 h incubation, 48 h enrichment expanded the detection limit of the test by 10 fold (from 14 CFU/ml to 1.4 CFU/ml), thus 48 h enrichment is used in our current diagnostic services. Available canine GAPDH gene sequences were used to design a Taqman assay to serve as an internal control to monitor DNA extraction efficiencies, and potential inhibition to PCR reactions for each sample. Addition of the canine GAPDH gene into the assay formed the new triplex qPCR, and it does not affect the sensitivity or specificity of the assay. Testing on 1915 canine blood samples demonstrated that the canine GAPDH gene was detected in all samples without exception indicating a very high coverage of the test over different canine breeds, and from different regions in the US. Analyzing a subset samples for the GAPDH gene resulted an average Ct of 18.9, and a mean Ct of 18.6. Most samples have a Ct between 17 and 22. Any sample with GAPDH Ct > 25 were re-extracted, and the newly extracted DNA and its 1/10 dilution were tested again by the qPCR assay. This method has resolved extraction issues, or PCR inhibitions in all cases, although not too many, that had GAPDH Ct > 25.

019

*Actinobacillus pleuropneumoniae* (APP) ApxIV toxin antibody ontogeny in serum, oral fluid and fecal specimens from animals inoculated under experimental conditions.

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Objective: APP ApxIV toxin is unique to APP and is expressed by all APP serotypes. For this reason, the detection of anti-ApxIV serum antibodies has been used to identify APP infections. The goal of this project is to describe ApxIV antibody kinetics in serum, oral fluids, and fecal specimens from animals inoculated under experimental conditions. Herein we describe initial findings on serum antibody responses using the APP LPS ELISAs (University of Montreal) and ApxIV ELISA (IDEXX Laboratories, Inc). Methods: 3 groups (6 pigs/group) of 14-week-old pigs were exposed to APP serovars 1 (ATCC 27088), 5 (ATCC 33377), or 7 (ATCC WF83) intranasally (2 ml) and by direct application (3 ml) to the tonsils using a challenge inoculum containing  $1 \times 10^6$  CFU/ml. Animals were housed individually throughout the experiment for the purpose of collecting individual pig oral fluid and fecal samples. Pigs were monitored for clinical signs daily throughout the entire experiment. Oral fluids samples were collected daily, blood samples were collected weekly, and fecal samples were collected every 3 and 7 days for 56 days post inoculation (DPI). Results: No clinical signs were observed in animals inoculated with serotype 5. In contrast, animals inoculated with serotypes 1 and 7 exhibited notable-to-marked clinical signs and were treated with antimicrobials, as necessary. Clinical signs included coughing, expulsion of mucopurulent respiratory secretions, vomiting, lethargy, anorexia, and reluctance to rise. Oral fluid collection was difficult with clinically-affected animals. One animal inoculated with serotype 1 died at DPI 3. Antibody responses were positively associated with the strength of the clinical response, i.e., both LPS and ApxIV antibodies were detected earlier (by DPI 7) and at higher levels in clinically-affected animals (serotypes 1 and 7). In pigs inoculated with serotype 5, LPS ELISA responses were delayed (DPI 14-35), low, and sometimes transient, with some animals testing negative before the end of the observation period (56 DPI). Likewise, ApxIV ELISA responses were low or absent in this group.

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021

Prevalence, genotypes, and risk factors for *Clostridium perfringens* among Ontario broiler chicken flocks

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*Clostridium perfringens* is the bacterium responsible for necrotic enteritis, an economically significant disease that occurs in broiler chickens. The objectives of this study were to 1) determine the prevalence and genotypes of *C. perfringens* in a representative sample of Ontario broiler chickens flocks; and 2) investigate the risk factors associated with *C. perfringens* and current on-farm biosecurity and management practices. Five pooled samples of caecal swabs from 15 birds per flock from 231 randomly-selected flocks were anaerobically-cultured using standard techniques for *C. perfringens*. Polymerase chain reaction (PCR) was used to test isolates for genes encoding various toxins ( $\alpha$ ,  $\beta$ ,  $\epsilon$ ,  $\iota$ , enterotoxin, and  $\beta$ 2), and real-time PCR was used to test isolates for the *netB* gene. Flock- and farm-level data were collected from producers through face-



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to-face interviews, and a mixed logistic regression model with a random effect for flock was used to identify risk factors. *Clostridium perfringens* was isolated from 181 of 231 flocks (78.4%). All isolates were type A, except for one type E. The *netB* gene was identified in 71 of 231 flocks (30.7%) and in 169 of 629 *C. perfringens*-positive isolates (26.9%). Risk factors associated with *C. perfringens* included ammonia levels greater than 25 ppm or notable nose/eye irritation at any time during grow-out compared to low ammonia levels (Odds Ratio (OR) = 4.04,  $p = 0.024$ ), never/sometimes using dedicated footwear when entering the restricted area during grow-out compared to always (OR = 6.18,  $p = 0.026$ ), not using an ionophore antimicrobial in the feed compared to using an ionophore antimicrobial in the feed (OR = 23.91,  $p = 0.003$ ), and feed mills B (OR = 19.20,  $P = 0.001$ ), D (OR = 8.77; 95% CI: 1.46 to 52.60,  $p = 0.017$ ), E (OR = 35.61, 95% CI: 4.33 to 293.21), F (OR = 11.87,  $p = 0.006$ ), G (OR = 6.77,  $p = 0.012$ ), and I (OR = 35.71, 95% CI: 4.03 to 316.11,  $p = 0.001$ ) compared to feed mill A. This study has shown that *C. perfringens* type A was highly prevalent among Ontario broiler chicken flocks and that the *netB* gene was present in a moderate proportion of flocks; factors associated with *C. perfringens* include antimicrobial use, management practices, and biosecurity protocols.

022

Seroprevalence and risk factors for *Coxiella burnetii* exposure in Ontario sheep and goats

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*Coxiella burnetii* is a zoonotic pathogen that causes abortion in small ruminants, and Q fever in humans. A cross-sectional study was performed to determine the seroprevalence and factors associated with *C. burnetii* exposure in sheep and goats in Ontario, Canada. Between August 2010 and February 2012, 4559 blood samples from 148 farms (22 dairy sheep, 50 meat sheep, 42 dairy goat and 34 meat goat) across Ontario were collected and tested for presence of *C. burnetii* antibodies using the CHEKIT Q fever kit (IDEXX Laboratories). Data on farm demographics and management practices were collected by questionnaire and used to construct a mixed multivariable model of animal serological status.

Exposure to *C. burnetii* was common in both species, in both the dairy and meat sectors. The sheep-level seroprevalence was 14.7% (347/2363), and 48.6% of farms (63.6% of dairy sheep and 42.0% of meat sheep) had at least one seropositive animal. Goat-level seroprevalence was 32.5% (714/2195), and 63.2% of farms (78.6% of dairy goat and 44.1% of meat goat) had at least one seropositive animal.

In a mixed logistic model with farm as a random effect, the following factors were associated with individual sheep seropositivity: positive association - female herd size (log scale), return of loaned sheep, and ewes sometimes lambing in separate airspace from the rest of the flock (vs. never); negative association - lambing pen cleaning practices. Additionally, the following main effects were associated with individual goat seropositivity: positively associated - breeding female herd size (log scale), does always or sometimes kidding in an airspace separate from the rest of the herd (vs. never), and presence of sheep or goat farms within a 5-kilometer radius; negatively associated - disinfection of kidding pens, breeding male herd size (log scale), and kidding outdoors in the absence of pigs on farm.

These findings support the importance of biosecurity practices and can be used to inform prevention and control strategies to reduce the risk of seropositivity to *C. burnetii* in sheep flocks and goat herds.

023

Identifying cattle farms and areas for risk-based surveillance for bovine tuberculosis in Minnesota

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In this study we propose to develop a risk-based surveillance system for MN cattle herds by creating a risk profile of each import cattle movement to the state of MN. We hypothesize that by doing so, we will identify high risk herds and zones for BTb introduction into the state of MN, which can direct resource allocation for the state animal health agency.

Data on import interstate cattle movements was obtained from the Minnesota Board of Animal Health for the years 2009 and 2011. Descriptive analysis was performed for 2009 and 2011. Movement data was summarized at the premise and county level and for both levels the distribution of cattle moved and number of movements was evaluated. In order to develop a strategy for a targeted surveillance system at the herd level for bovine tuberculosis in MN, each import movement was risk profiled based on known risk factors. The data was analyzed by fitting a linear regression model at the county level, to determine which variables were affecting higher number of movements at the regional level.

In each year, about 1500 herds had import movements to MN. Most of the cattle imported were in the categories of beef and feeder cattle, mostly originated from bordering US states. The peak season for incoming cattle and import movements was the fall season for both years. The risk model identified four risk groups with about 500 (~2% of total cattle farms in MN) cattle premises in the Very High and High risk group for each year. The southeast and southwest zones of the state had the highest density of cattle premises with movements and also cattle premises in the higher risk groups.

In this abstract an approach for risk-based surveillance for BTb is presented using available data from a state animal health agency, which outlines a method that can be used in the US for BTb and other disease scenarios.

024

Detection of *Salmonella enterica* in the dairy environment using a commercially available lateral flow immunoassay.

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*Salmonella enterica* is one of the most common causes of health care-associated infections in veterinary hospitals. Its control is often dependent upon rapid detection; however, standard detection methods may take up to 3-5 days depending on the type of sample and test being employed. The purpose of this study was to describe the sensitivity and specificity of a rapid, point-of-care, commercially available lateral flow immunoassay (LFIs) for detection of *Salmonella enterica* in environmental samples.

Environmental samples were collected from high traffic areas in dairy operations in Colorado and tested in parallel using standard aerobic culture and LFIs. Each environmental sample was pre-enriched in 90mls buffered peptone water for 18hrs at 43°C, then 1 ml was passed into 10ml of tetrathionate broth for 18hrs at 43°C, and plated on XLT4 for 18hrs at 43°C. Enriched cultures were also tested using LFIs.

Overall, LFIs were not as sensitive as aerobic culture for the detection of *S. enterica* in environmental samples. Out of 190 samples, 106(56%)

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were culture-positive and only 38(20%) were test-positive by LFIs. In general, LFIs showed a limited ability to detect isolates that were serogroup C1 and K. Upon stratified analysis, among farms with isolates that were not C1 or K, 43 of 67 samples (64%) were culture-positive and 38 (57%) were test-positive by LFIs.

The findings of this study suggest that detection rates for enriched culture and LFIs are comparable but may be farm-specific. Further testing is needed to determine the effect of strain and/or serotype on the utility of these test strips in clinical applications.

025

Improved characterization of *Salmonella enterica* shedding among reptile patients at the James L. Voss Veterinary Teaching Hospital

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In the U.S., an estimated 6% of approximately 1.2 million sporadic human *Salmonella enterica* infections annually are attributed to reptile or amphibian exposure. Reptiles continue to be relatively popular pets posing a significant public health risk. Sample collection and isolation poses several challenges, including difficulty in patient handling, intermittent shedding, and insensitive culture methods. The goal of this study was to improve the characterization of *Salmonella* shedding among reptilian patients, determining shedding prevalence, assess sampling methods, and compare sensitivity of detection methods.

A cross-sectional study of reptile and chelonian patients was undertaken at the CSU-VTH. Each patient had a cloaca swab cultured in TET for 18hrs at 43°C and plated on XLT4 for 18hrs at 43°C; and a body Swiffer sample pre-enriched in BPW for 18hrs at 43°C, then passed into TET for 18hrs at 43°C, then plated on XLT4 as above. Environmental Swiffer samples were also collected before and after exam table cleaning and disinfection, pre-enriched in BPW for 18hrs at 43°C, then passed into TET for 18hrs at 43°C, then plated on XLT4 as above. All samples were also tested via PCR and, for each body Swiffer sample, tested using a lateral flow immunoassay.

Approximately 45 reptiles and chelonians that were at least 30 grams by weight were enrolled in this study. All had a cloacal swab and a body Swiffer sample (sampling approximately 80% of the body surface including dorsum, ventrum and feet) collected. All samples were tested in parallel with enriched aerobic culture and PCR.

Reptiles and chelonians present a potential source for environmental contamination and infections among animals and people. The results of this study provide evidence that will allow for prevention efforts to be tailored to the hospital as well as home environment.

026

Biosecurity, a biography: ten years of hard lessons.

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The veterinary hospital missions means that animals clinically affected by agents with potential to spread among hospital cases, as well as subclinical carriers that may go unrecognized, are always likely to be present. Moreover, hospitalized animals differ from the general population in that they are more likely to shed or acquire infectious agents because they are subject to known risk factors for infections of various types. The standard of care at every veterinary hospital should include scrupulous hygiene, awareness of the dangers of transfer of infectious agents between both animals and people, and procedures to reduce infection risk. The George D. Widener Hospital at New Bolton Center is a critical care referral center and among the busiest large animal hospitals in North America. In 2004 a serious outbreak of salmonellosis caused the hospital to close to all admissions for 3 months. In response to this outbreak a commitment to biosecurity was made at the school's highest level, dedicated faculty and staff positions with sole responsibility for infection control were created, and a proactive, evaluative infection control program (ICP) was initiated. Based on evidence gathered over the last 10 years the ICP has undergone significant evolution. Stages in the evolutionary process and the evidence used to direct this maturation will be reviewed. Issues that will be addressed include: financing, cost to benefit considerations, roles of both environmental and active and passive patient surveillance, stakeholder acceptability, facilities design, protective apparel, education and training. While there is no interchangeable "one-size-fits-all" ICP, certain aspects of biosecurity should be considered by every veterinary hospital. Although our ICP might be more extensive than is necessary for many hospitals, lessons from our experience are relevant to all.

027

Evaluation of POKKIT, field deployable technology, for molecular based detection of Foot and Mouth Disease virus, Classical Swine Fever virus and African Swine Fever virus.

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Rapid detection and response to high impact infectious disease outbreaks is paramount to mitigate economic and animal losses. Validated technology is extremely important since false negatives may lead to delayed responses which can be costly. Moreover, proper training, testing oversight and results reporting when considering device deployment are equally important. With these key concepts in mind, we developed a 5 stage validation pipeline for evaluation of candidate deployable devices. Pipeline stages include Stage 1: target selection, Stage 2: reproducibility and Limits of detection (LOD) determination, Stage 3: analytical sensitivity and specificity, Stage 4: field trials and training programs and Stage 5: translation. Preliminary results of experiments for stage one and two of this evaluation pipeline for Foot and Mouth Disease virus (FMDv), Classical Swine Fever virus (CSFv) and African Swine Fever virus (ASFv) field detection will be presented. Insulated isothermal PCR (iiPCR) assays were developed for the portable device, POKKIT (GeneReach USA). LOD100 for these assays was determined on the portable device as compared to the USDA reference assays utilizing non-infectious controls (Plum Island phage-based control-PIPC) provided by collaborators at the Plum Island Animal Disease Center. LOD for reference assays were determined on the BioRad CFX 96 using Applied Biosystems Path ID RT-PCR master mix and Quanta qScript XLT RT-qPCR Toughmix for CSF and FMD and Quanta qPCR Fast mix II for ASF. LOD100 for reference assays is between 2-20 copies for FMD and 3.2 copies for CSF. LOD100 for ASF is one copy. Assays run on the POKKIT yielded similar sensitivities with LOD100 between 100-20 copies for FMD, 3-30 copies for CSF and 1-10 copies for ASF.

Satisfactory results in this stage of the evaluation pipeline warrant further evaluation of these reagents using virus stocks and samples from

## Biosafety and Biosecurity

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infected animals. Preliminary data demonstrate the future utility of portable PCR devices for accurate and sensitive detection of high impact pathogens under field conditions.

028

Using social network analysis to understand epidemic potential in equine populations: a pilot study.

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Horses travel significant distances in order to participate in show and sporting events. Contact with new locations and horses while participating in sporting activities presents challenges for the prevention and control of pathogens within these populations. There is little information available regarding the connectivity of horses that would allow for the assessment of epidemic potential within equine populations in Ontario. The objective of this pilot study was to collect data that could be used to visualize the network of potential contacts resulting from a single equestrian show in order to understand the implications of infectious disease transmission within the population. Horse show participants completed a survey asking about their horse's "home facility" and infection control protocols, previous and future travel patterns, and vaccination status. Slightly more than half of the horses in attendance (54%) travelled < 50km from their home facility to attend the show (range: 6.0 km - 370.0 km). Seventy-seven per cent (77%) of respondents indicated that there were other horses from their horse's home facility also present at the event. The most common vaccinations amongst horses in attendance were equine influenza (EI), rabies, and West Nile Virus, with 86% of horses vaccinated against all three viruses. Most participants (75%) followed standard infection control protocols while at the show, including the use of different equipment, cleaning tools, and water/feed between horses. The results of the study are being used to construct network diagrams of the horses attending the event, as well as disease transmission models to simulate the potential impact of the introduction of different equine infectious diseases to the network. Understanding the interaction between network structure and disease dynamics will aid in identifying effective strategies for disease surveillance and allow us to optimize our responses to the potential introduction and spread of equine infectious diseases.

029

Assessing spatial dispersal of pathogens around human settlements

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Purpose: Both humans and livestock alter the environment around them. One way in which they alter their environment is by spreading pathogens around the places they frequent. These pathogens can then be transmitted to other humans and animals and cause disease. It is unclear to what extent this increases the risk for contact with pathogens in environments surrounding human habitations, but biosecurity practices must be designed to combat this risk.

Methods: Survey, GPS, and environmental soil samples were taken from 70 households amongst 14 villages in the Adamawa Region of Cameroon. We analyzed these data in ArcGIS to create risk maps providing a more keen understanding of the movement of people and their animals. Associated with these maps, we traced the source of soil contamination in different locations as well as the quantities of pathogens (STEC) and antimicrobial resistance (tetQ gene).

Results: Free grazing cattle are the most mobile animal species in the region, while men and adolescents are the most mobile humans. Long range movements (outside of the region) are rare events for this population, but do provide a source of risk. Based on survey results, oxytetracycline is the most commonly used antibiotic in animals, but results of spatial distribution of tetQ were still pending at the time of abstract submission.

Conclusions: These data describe the spatiotemporal relationship between humans and livestock and the presence of fecal contamination among cattle ranches, wildlife habitations, and villages in the Adamawa Region of Cameroon. Knowing the spatial distribution of risk will help in designing biosecurity measures to prevent transmission of human and animal pathogens through the environment.

030

Survey of Iowa residential bats for influenza viruses

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Bats have a unique role in harboring and spreading of a myriad of pathogens, including coronaviruses, filoviruses, henipaviruses, and lyssaviruses. Recent reports of influenza A virus in bats residing in Guatemala and Peru have raised further concerns of them being a potential reservoir for influenza viruses and their potential to transmit these viruses to other animals and humans. The present study was designed to carry out a diagnostic laboratory-based regional surveillance of North American bats for influenza viruses. A total of 125 live or freshly dead bats received at Iowa State University Veterinary Diagnostic Laboratory (ISUVDL) during 2012-2013 were tested using PCR-based assays for influenza A, B, C and D viruses, including bat specific strains (H17N10 and H18N11) of influenza A virus. Briefly, tissue samples from each bat were divided into lungs and intestines, homogenized, underwent nucleic acid extraction, and tested using real-time or conventional RT-PCR assays. No evidence was found that any of the tested bats harbored influenza A, B, C or D viruses. However, our findings are based on a small, regional subset of bats received at ISUVDL and may not represent the diversity of bat population across North America. Continued surveillance studies on various influenza viruses in bats are warranted.

## Companion Animal Epidemiology

031

Scrotal castration as a safe and effective means of male canine sterilization

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## Companion Animal Epidemiology

(031 continued)

For years, a prescrotal surgical approach has been the only accepted method of male dog sterilization, as dogs are considered to be “scrotal conscious.” The prevailing thought has been that a scrotal incision will cause more complications including swelling and induction of self-trauma. The focus of this study is to evaluate the hypothesis that there are no differences between the prescrotal and scrotal technique. In a collaborative effort between Mississippi State University College of Veterinary Medicine (MSU-CVM) and Humane Alliance, 437 apparently healthy male dogs over the age of 6 months were randomly allocated by coin toss into 2 treatment groups and castrated by either a prescrotal (n=206) or scrotal incision (N=231). Complications were recorded up to 72 hours following the procedure by individual owners and humane organization employees. At MSU-CVM the length of the procedure was recorded to track the difference in efficiency. Complications were categorized by the presence or absence of hemorrhage, self-trauma, pain and swelling. Data were analyzed for surgical efficiency and the presence or absence of complications. Multivariable logistic regression was used to measure the strength of association between the occurrence of each of the complications and the explanatory variables of method, institution and weight of dog. Differences in duration of surgery by method were assessed by analysis of variance. A p value < 0.05 was considered statistically significant for analysis.

The method of castration was not found to be significantly associated with hemorrhage, pain or swelling. Fifty-four animals (prescrotal = 34, scrotal = 20) were recorded as inflicting self-trauma through biting, licking or chewing their incision. The odds of self-trauma were 1.96 times greater (p=0.04) in animals undergoing the prescrotal method than in those castrated by the scrotal method. Scrotal castration was faster than prescrotal castration and had similar or reduced incidence of post-operative complications. This study supports scrotal castration as a safe and efficient method of male canine sterilization.

032

Factors associated with hematuric struvite crystalluria in cats evaluated at general care veterinary hospitals in the United States (2007-2011)  
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Purpose: To identify demographic and clinical factors associated with diagnosis of hematuric struvite crystalluria in cats seen at general care veterinary hospitals in the United States and to compare these factors with those identified previously for struvite urolithiasis.

Methods: Electronic medical records of all cats evaluated at 790 general care veterinary hospitals in the United States between October 2007 and December 2011 were reviewed to identify cats that developed hematuric struvite crystalluria (n = 4,032) and control cats with neither history of hematuria nor crystalluria (n = 8,064). Demographic information extracted included diet, age, sex, neuter status, breed, hospital location, and date of diagnosis. Clinical information extracted included urinalysis results, and history of cystitis and diabetes mellitus. Potential risk factors were assessed with univariable and multivariable logistic regression analyses.

Results: Hematuric struvite crystalluria in cats was significantly (p < 0.05) associated with a positive diagnosis of cystitis, and males had significantly higher odds of this condition than females. Urinary factors significantly associated with this condition were the presence of the following: basic (vs neutral) pH, white blood cells, bacteria, casts, protein concentration > 30 mg/dL, and ketone concentration ≥ 5 mg/dL.

Conclusions: Hematuric struvite crystalluria and struvite urolithiasis in cats share similar urinary risk factors. However, males had significantly higher odds of the former condition whereas females were at higher risk of the latter. Hence, the diagnosis of hematuric struvite crystalluria does not necessarily predispose cats to struvite urolithiasis. Overall, efforts that prevent cystitis in cats may be useful in reducing hematuric struvite crystalluria.

033

Prevalence and association with Feline Upper Respiratory Disease severity for the detection of select pathogens and risk factors in Midwestern animal shelter cats

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Feline Upper Respiratory Disease (FURD) spreads rapidly in cats housed in animal shelters and is a leading cause of morbidity and mortality. A cross sectional study was performed to determine the prevalence of selected pathogens and to assess whether detection of these pathogens and other risk factors were associated with FURD. This study included 190 cats (71 with FURD and 119 without) from five animal shelters and one veterinary teaching hospital in Iowa and Nebraska during 2011-2012. Ocular, oral, and nasal swabs were tested using quantitative real time polymerase chain reactions for Feline Herpesvirus (FHV), Feline Calicivirus (FCV), *Chlamydomydia felis* (*C. felis*), *Bordetella* species, *Mycoplasma felis* (*M. felis*), and other *Mycoplasma* species. Prevalence of FHV and *M. felis*, were highest at 49.0% and 40.0%. Prevalence of *Bordetella* spp., FCV, the *Mycoplasma gateae* cluster, and other *Mycoplasma* spp. were 24.7%, 23.7%, 19.5%, 15.3% respectively. Prevalence of *C. felis* was lowest at only 3.2%. Multivariate modeling, performed for all pathogens and risk factors, demonstrated a significant relationship between the detection of *Bordetella* spp. (Odds Ratio (OR) = 3.143) and FCV (OR = 2.830) and increased respiratory disease severity. Cats housed in shelters for 2-6 months had significantly reduced severity of respiratory disease (OR=0.146) as compared to those housed for less than one month. FHV and *M. felis* were endemic in the study populations and the lack of association with severe disease may be attributed to mild or early infections, long term exposure, maturity of the immune response, or latent infections and carrier status. Study results suggests that management of FURD in shelter cats in this region should focus on mitigation of acute *Bordetella* and FCV infections in cats through the use of clinical microbiology and appropriate antimicrobial therapy for *Bordetella*. Minimization of transmission of *Bordetella* can be performed through separation of canines and felines and biosecurity protocols which limit interspecies transmission of *Bordetella*. The spread of FCV can also be minimized through the prompt isolation of cats exhibiting FURD clinical signs.

## Companion Animal Epidemiology

034

An insulated isothermal PCR-POCKIT™ method for rapid and sensitive detection of canine parvovirus at the point of care

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Canine parvovirus type 2 (CPV-2) is a highly contagious pathogen of both domestic and wild animals, producing high mortality rates in young animals. Recently, a field-deployable POCKIT™ device, using insulated isothermal PCR (iiPCR), has been shown to provide a user-friendly, rapid molecular platform to facilitate point-of-need (PON) diagnosis of pathogens. An iiPCR-POCKIT™ method was developed for detection of CPV-2 in fecal samples. The CPV-2 iiPCR targets a conserved area in vp2 gene to detect all circulating strains of CPV-2 and feline panleukopenia. The 95% limit of detection (LoD) of the assay was determined using 10-fold serial dilutions of plasmid DNA. Specificity evaluation was performed with canine adenovirus 2, canine herpesvirus, canine parainfluenza virus, canine enteric coronavirus, canine distemper virus, Salmonella enterica, and Clostridium perfringens. Assay sensitivity and performance in detecting clinical samples were compared with an in-house TaqMan based real-time PCR (University of Tennessee Veterinary Medical Center -UTVMC). Sensitivity comparison was performed using serial dilutions of a CPV-2b strain. A total of 100 fecal samples from dogs with signs suggestive of CPV infection submitted to the UTVMC Clinical Virology Lab from 2010 to 2014 (including CPV-2b and 2c strains) and a feline panleukopenia strain were tested for performance comparison. The 95% LoD of the iiPCR was 13 copies of plasmid DNA. Comparison between iiPCR and real-time PCR revealed equivalent sensitivity in detecting CPV DNA. There was no detection of the other canine pathogens examined. Two of the 63 positive canine samples by real-time PCR tested negative by iiPCR. All 37 real-time PCR negative samples also tested negative by iiPCR. Excellent agreement ( $k = 0.96$ ) was found between the two assays, with sensitivity of 96.88% (CI = 91.16-100%) and specificity of 100% (CI = 93.18-100%). The iiPCR also detected the feline panleukopenia strain. In conclusion, the iiPCR was highly specific and sensitive for the detection of CPV-2 in fecal samples. This iiPCR-POCKIT™ method is a useful portable diagnostic tool for PON identification of CPV-2.

035

Molecular epidemiological analysis of MRSP environmental contamination in a veterinary hospital: Looking beyond infections and treatments

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Methicillin-resistant *Staphylococcus pseudintermedius* (MRSP) is an emerging canine pathogen associated with ear, skin and postsurgical infections in dogs. In veterinary hospitals, the development of infectious disease prevention and control programs is critical for the management of such nosocomial pathogens. However, epidemiological information regarding MRSP beyond the clinical point of view (treatment), necessary to build such programs, is limited. Therefore, an active surveillance program was established at large veterinary teaching hospital between 2007-2013 to understand the molecular epidemiology and ecology of MRSP in a hospital environment. We hypothesized that if MRSP is present in canine patients treated at this practice, then this bacterium could contaminate and survive on multiple surfaces across the hospital overtime. Moreover, patterns of contamination and “hot spot” surfaces could be identified. Molecular epidemiological analysis was performed on environmental and canine-origin MRSP isolates obtained in this period. Antimicrobial susceptibility testing, SCCmec typing, PFGE typing, and dendrographic analysis were used to characterize and analyze these isolates. Overall, 9.1% of the surfaces and 11.8% of the canines sampled were MRSP positive. Phenotypically, over 90% of the isolates obtained were classified as multidrug resistant. Genotypically, the majority of the MRSP strains carried SCCmec type II-III and V. Dendrographic analysis showed high diversity, with a constant introduction of various clones into the hospital environment. However, movement or spreading of MRSP clones throughout different areas of the hospital and survival overtime (consecutive months) was rarely observed. Interestingly, the most contaminated surfaces were examination lamps (24.1%), computers (15.0%) - both of them considered human contact surfaces-, and gurneys (14.1%). Based on these results it is clear that MRSP frequently contaminates commonly touch surfaces, and patients are in direct contact with many of them. These findings highlight the need of steady and effective disinfection protocols targeting surfaces that are regularly contaminated with MRSP.

036

Use of patient body temperatures in surveillance for healthcare-associated infections in a veterinary hospital.

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There is an ethical and legal responsibility to optimize infection control programs in all hospitals, and surveillance is a key component of these efforts. Syndromic surveillance has been proposed as an efficient tool to screen for infection control problems and these efforts can be facilitated by increasing utilization of electronic medical records (EMR) and other information management systems. Fever is frequently a sequella of infection and rectal temperatures are typically assessed with every physical examination. As such, the purpose of this study was to evaluate use of rectal temperature data obtained from hospitalized patients as an efficient and effective tool to assist with surveillance for issues related to healthcare associated infections (HCAI) in veterinary patients.

Data for rectal temperatures and patient information that were recorded in EMR for a veterinary teaching hospital were abstracted from 1/1/2012 to 7/15/2014. This included a total of 43,059 visits to the hospital, during which 6.1% (2,621) of patients were identified as febrile. For small animal (SA; canine and feline) visits, 3.5% (1232/35,562) of patients were febrile, compared to 19.2% (678/3,523) of patients during equine visits and 21.9% (1157/5293) of visits for livestock animals (LA; camelid, bovine, caprine, ovine and porcine). A total of 109,692 rectal temperatures were recorded, of which 5.9% (6,517) were febrile. Among these, 3.0% (2,441/80,466), 10.2% (2,475/24,162), and 23.3% (1,025/4,407) of individual temperature measurements were febrile for SA, equine, and LA patients, respectively. Febrile events were analyzed to identify associations with hospital and patient factors.

This study provides an initial examination of trends in febrile temperatures recorded among hospitalized veterinary patients. Deviations from these baseline trends may indicate an increase in nosocomial infections, allowing for prompt implementation of infection control measures. Further, once infection control measures have been implemented, a return to the baseline would indicate an effective protocol. This information would be vital for the establishment of protocols based on successful outcomes.

## Companion Animal Epidemiology

037

Non-fatal injury occurrence in southern California Thoroughbred racehorses 2009-2010

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Among racing and training Thoroughbreds, musculoskeletal injuries are the most common cause of death or euthanasia, the most common cause of exiting training, and are also a leading cause of wastage. The primary objective of the current study was to describe the incidence, anatomic distribution and characteristics of non-fatal injuries in Thoroughbred racehorses as diagnosed by private practice veterinarians in Southern California. Participating veterinarians recorded non-fatal injuries (definitive diagnosis of a musculoskeletal condition resulting in lameness, injury or loss of training > five days) incurred by Thoroughbred racehorses in their care. Secondary objectives were comparison of this private-practice recording system with an existing regulatory system, and comparison of fatal and non-fatal injury rates. Between May 1st 2009 and April 30th 2010, non-fatal injuries were recorded by seven veterinarians in four practices. Non-fatal injuries were diagnosed in 2-4% of horses in race training per month. The majority of injuries were acute rather than chronic. Stress fractures, superficial digital flexor tendonitis, and suspensory ligament desmitis were common. Agreement between non-fatal injuries recorded in the current study and those recorded via an existing regulatory system was poor, with neither system capturing all injuries. Non-fatal injuries occurred much more often than fatal injuries. Non-fatal musculoskeletal injury remains an ongoing issue for Thoroughbred racehorses, and an accurate, comprehensive system for recording these injuries is needed.

038

Factors associated with long-term survival of geriatric horses in the U.S.

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In the U.S., geriatric horses account for approximately 7.6% of the equine population - a population that continues to increase with improvements in veterinary care. Currently, limited information exists on factors contributing to long-term survival (at least 1-year) of this subpopulation of horses, which may be critical to veterinarian and owner decisions to pursue treatment. The purpose of this study was to determine factors associated with long-term survival of geriatric horses in the U.S.

A retrospective cohort study was performed with 248 geriatric horses (horses  $\geq 20$  years of age) admitted to the CSU-VTH during 2012; and 248 non-geriatric horses ( $\geq 2$  and  $< 20$  years of age) randomly selected from all adult horses admitted in 2012. Data from electronic medical records were extracted for exposure variables of interest including presenting complaint, physical examination findings and results of diagnostic testing. An owner questionnaire was conducted via email to collect information on horse status (alive or deceased), reason for death (if applicable), if the horse returned to intended use, and general geriatric horse management and care. Multivariable logistic regression was used to determine factors that may be associated with long-term survival of geriatric horses (yes / no).

During the study period there were approximately 2121 adult equine primary visits. Of which, 248 were classified as geriatric horses (ages ranged from 20 - 41.4 years) and 1873 were non-geriatric horses (ages ranged from 2.2 - 19.9 years).

Factors in this study determined to be associated with long-term outcome in the geriatric horse population will provide prognostic information for veterinarians and owners when managing this growing subpopulation of horses.

039

Canine B-cell chronic lymphocytic leukemia shows strong breed-specific risk

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B-cell chronic lymphocytic leukemia (B-CLL) is a hematopoietic neoplasm characterized by an expansion of small, mature B cells. In humans, one of the primary risk factors for developing CLL is genetic; first-degree relatives of CLL patients have an 8.5-fold higher risk for developing the disease. In dogs, it is conceivable that genetic risk manifests as a breed predisposition, but no prior studies have directly assessed this. This study aims to further describe canine B-cell CLL by providing a systematic description of the typical patient presentation, associated clinical signs and breed predisposition.

We conducted a retrospective cross-sectional study using samples submitted to the Colorado State University Clinical laboratory for immunophenotyping between September 2010 and June 2014. Cases were dogs with  $>5,000$  lymphocytes and a homogeneous expansion of small CD21+ lymphocytes on flow cytometry. Signalment, physical exam (PE) findings, and CBC results at the time of diagnosis were obtained as part of the standard submission form.

Overall, 8% (491/6,155) of submissions for suspicion of lymphoproliferative disease (LPD) fit our criteria for diagnosis of B-CLL. On average, dogs were diagnosed at 11 years of age and males and females were equally represented. Among cases where PE findings were reported, approximately half presented with peripheral lymphadenopathy or splenomegaly. Compared to mixed breeds, twelve breeds had significantly increased odds of CLL, almost all of which were small breeds. Notably, English Bulldogs had a unique presentation of the disease, diagnosed at a median of 6 years of age, with significantly lower Class II MHC and CD25 expression.

Canine B-CLL appears to be overrepresented in certain breeds. Future genetic studies focusing on these breeds may be useful in localizing the genes responsible for this disease. In addition, the unique presentation of English Bulldogs provides evidence that there may be more than one form of this disease. Further studies are necessary to determine whether presenting signs are associated with survival.

040

Multicenter case-control investigation of risks associated with development of canine lymphoma in North America: 18,826 cases (1990-2009)

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Purpose: Canine lymphoma (CL) is considered to be a valid model for the study of non-Hodgkin's lymphoma in humans. The incidence of NHL has increased worldwide since the 1970s, yet the causal factors for this disease remain largely unknown in both species. The purpose of this study

## Companion Animal Epidemiology

(040 continued)

was to investigate associations between breed and other intrinsic factors and the occurrence of CL in order to determine the most appropriate dog breed model for use in NHL research.

**Methods:** This retrospective case-control study included dogs (n=67,712) admitted to one of 23 veterinary teaching hospitals in North America. Cases were defined as dogs diagnosed with CL between 1990 and 2009, as identified using the Veterinary Medical Database (VMDDB). A comparison group was randomly selected from a restricted group of dogs diagnosed with any musculoskeletal condition in the same population of dogs. The association between intrinsic factors and the diagnosis of CL was estimated using logistic regression models.

**Results:** There is inherent risk associated with dog breed and the outcome of CL with some breeds, such as Scottish terriers, being at increased risk of developing disease and other breeds, such as Dachshunds, being at low risk of developing disease. Neutered dogs are at increased risk of developing CL as compared to intact female dogs.

**Conclusions:** The proportion of cases of CL diagnosed in dogs at VTHs has increased, which is similar to trends seen in the human population. Identification of individual dog breeds with an increased risk of developing CL offers the opportunity to localize genetic abnormalities that have been difficult to identify in human populations.

041

Differences in the geographic distribution of B-cell and T-zone lymphomas in Golden retrievers in the United States

**A. Ruple**, A. Avery, P. Morley; Colorado State University, Fort Collins, CO, USA.

**Purpose:** Malignant lymphomas in both dogs and humans are a heterogeneous group of diseases. In dogs, it has been shown that some breeds develop B-cell or T-cell derived lymphoma with differing frequencies, suggesting there is heritable risk for developing specific subtypes of canine lymphoma (CL). The purpose of this study was to examine differences in the geographic distribution of two distinct subtypes of CL, B-cell lymphoma (BCL) and T-zone lymphoma (TZL), in Golden retrievers in the United States.

**Methods:** A total of 454 Golden retrievers diagnosed with either BCL or TZL using flow cytometry at the Clinical Laboratory at Colorado State University (CSU-CI) were included in this cross sectional study. Samples submitted to the CSU-CI between January 1, 2007 and April 30, 2014 were analyzed using a Coulter XL flow cytometer. Subjects were categorized according to the zip code location of the veterinary hospital which submitted the sample, using the US Census Divisions. Associations between geographic areas of the US and the phenotypic variant of lymphoma diagnosed were examined using multivariable logistic regression.

**Results:** There is a difference in the geographic distribution of BCL and TZL subtypes of CL diagnosed in Golden retrievers in the United States with dogs in the northeast (OR =3.4, 95%CI =1.6-7.0) and East North Central regions (OR =12.1, 95%CI =3.6-40.5) being more likely to be diagnosed with TZL as compared to dogs in the Mountain region of the US.

**Conclusions:** Future work in veterinary medicine related to etiologic investigations of CL should differentiate between specific subtypes of the disease.

## Ecology and Management of Foodborne Agents

043

Campylobacter jejuni isolated from cattle in Michigan: genetic diversity, antimicrobial resistance, and the impact on public health

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Campylobacter jejuni, a zoonotic pathogen, is the most common bacterial cause of human gastroenteritis in the world. With the increasing resistance to ciprofloxacin (CIP) and azithromycin (AZI), the drugs of choice for treatment, understanding the epidemiology and possible transmission from animal reservoirs has become essential. We studied C. jejuni isolates from cattle in Michigan to understand the antimicrobial resistance, genetic diversity and the potential impact on public health.

We isolated 90 C. jejuni from cattle in Michigan, and performed molecular genotyping by multilocus sequence typing (MLST) and repetitive sequence PCR (rep-PCR). To determine the antimicrobial resistance, the broth microdilution test was conducted using commercial plates (CAMPY, TREK diagnostics). For phylogenetic and statistical analysis, we used MEGA, Bionumerics and SAS.

Seventy (77.8%) of 90 cattle isolates showed resistance to one or more antimicrobials. Resistance to tetracycline (TET) was the highest (76.7%), followed by CIP (20%) and nalidixic acid (NAL) (20%). Twenty-two isolates (24.4%) showed multidrug resistance, with the major pattern being resistance to CIP, NAL, and TET. Seventeen sequence types (ST) were observed among 90 isolates by MLST; however rep-PCR identified 32 different patterns. Association between specific genotypes and antimicrobial resistance pattern was observed, and distributions of these strains were different among herds. When compared to our prior study with human C. jejuni isolates in Michigan, the same STs with identical antimicrobial resistance pattern were observed across species.

High prevalence of antimicrobial resistant C. jejuni was observed from cattle in Michigan. Molecular characterization of the isolates showed high genetic diversity of C. jejuni shed by cattle, but there was variation in the predominant STs across herds. There were overlapping strains found between cattle and humans, when characterized by MLST and antimicrobial resistance pattern, implicating cattle as important reservoirs for human C. jejuni infections in Michigan.

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An evaluation of the impact of litter chemical amendments on reducing Campylobacter jejuni in broilers

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Campylobacter jejuni persists in colonized broilers and is heavily shed in their feces, which leads to contaminating the bedding material (litter). Therefore, used litter may constitute an important source of infection of new flocks. Here, we investigated the impact of aluminum sulfate (Alu), sodium bisulfate (Sob), and magnesium sulfate (Mgs) on C. jejuni's survival in litter and cognate chicken colonization. In controlled microcosms, the application of Alu and/ or Sob significantly reduced the litter's pH for more than 14 days, while only the addition of Mgs reduced moisture by ~50%. The addition of Alu and/ or Sob in combination with Mgs significantly reduced the colony forming units (CFU) of a C. jejuni cocktail,

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consisting of 81-176 and 5 other chicken isolates, in the microcosms. Specifically, no *C. jejuni* isolates were retrieved after 24 h from the treated litter, while *C. jejuni* was still detected after 48 h in the untreated litter. Next, we performed pen trials using broilers and litter that was treated as follows: Pen 1: untreated (control); Pen 2: treated with Alu+Mgs; Pen 3: treated with Sob+Mgs; Pen 4: treated with Alu+Sob+Mgs. Our data showed that the chemical treatments did not adversely affect the weights of the birds. In chickens experimentally inoculated with the *C. jejuni* cocktail, the treatments did not significantly reduce *C. jejuni* colonization of the ceca. However, in a separate experiment using uninoculated chickens, the Alu+Sob+Mgs treatment significantly reduced the number of colonized birds and *C. jejuni* was not detected in the litter. Specifically, no cecal colonization was detected in 3-week old birds, while only 1 out of 10 birds tested was colonized with low numbers of *C. jejuni* at week 6. Metagenomic analysis showed that the Alu+Sob+Mgs treatment also affected the bacterial community composition of the litter. Notably, the treatment reduced the Firmicutes from 91% in the untreated litter to 79.5% and the Actinobacteria from 9% to 1%, while the Bacilli also decreased from 28% to 2.5%. Taken together, our data suggest that the treatments have the potential of providing a relatively efficient approach for controlling *C. jejuni* in litter.

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Summer and winter prevalence of O26, O45, O103, O111, O121, O145 and O157 Shiga toxin-producing *Escherichia coli* (STEC) in feces of feedlot cattle

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Shiga toxin-producing *Escherichia coli* (STEC) are foodborne pathogens of public health importance. Cattle, a major reservoir of STEC, shed these pathogens in their feces. Feces can contaminate cattle hides which can lead to contamination of carcasses at slaughter potentially leading to contamination of beef products. Many studies have shown serogroup O157, the most common STEC, to follow a seasonal pattern, with prevalence peaking during summer months; however, little is known about the seasonal prevalence in cattle of the six non-O157 STEC serogroups (O26, O45, O103, O111, O121 and O145) that are also considered adulterants in beef. The objective of this study was to determine the prevalence of seven STEC serogroups (O26, O45, O103, O111, O121, O145 and O157) and the virulence genes (*stx1*, *stx2* and *eae*) associated with these pathogens in feces of commercial feedlot cattle during summer (June to August 2013) and winter (January to March 2014) months. Twenty-four pen floor fecal samples were collected from 24 pens in both the summer and winter months at a large commercial feedlot in the central United States. All samples were subjected to culture-based detection methods that included serogroup-specific immunomagnetic separation and plating on selective media, followed by a multiplex PCR for serogroup confirmation and virulence gene detection. A sample was considered STEC positive if a recovered isolate harbored an O gene of interest, at least one Shiga toxin gene (*stx1* and/or *stx2*) and the intimin gene (*eae*). The cumulative unadjusted sample level prevalence of STEC O26, O103, O145 and O157 during summer (n=576) was 1.2, 1.7, 1.0 and 42.9%, respectively, whereas STEC O45, O111, and O121 were not detected. During winter months, none of the seven STEC were isolated from these cattle. The results of this study indicate that non-O157 STEC are rare, but generally follow the same seasonal pattern as O157 STEC. However, further research is needed to determine if this trend holds true across other cohorts of cattle, feedlots and regions of the country.

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Feedlot- and pen-level prevalence of Shiga toxin-producing *Escherichia coli* in feces of commercial feedlot cattle

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The objective of the study was to determine feedlot- and pen-level fecal prevalence of seven Shiga toxin-producing *Escherichia coli* (STEC) serogroups (O26, O45, O103, O111, O121, O145 and O157) and their associated virulence genes (*stx1*, *stx2*, *eae*, and *ehxA*) in feedlot cattle. Four commercial feedlots from each of two major cattle feeding states, Nebraska and Texas, and with pens of cattle on finishing diets during the summer were selected for sampling. Up to 16 pen-floor fecal samples were collected from each of 4 to 6 pens per feedlot per visit, with one feedlot per state visited once a month from June to August, 2014. Culture-based procedures included fecal enrichment in *E. coli* broth, immunomagnetic separation with individual beads for O157 and two sets of pooled beads for the six non-O157 serogroups, plating on selective medium, followed by polymerase chain reaction based methods for confirmation of STEC serogroups. Sampling and STEC confirmation are still underway, thus only preliminary serogroup-level data are reported here. Overall, feces from 100% of feedlots (n=8) and pens (n=104) had *E. coli* with one or more O group genes. Feedlot-level prevalence (at least one sample testing positive per feedlot) estimates were 100.0% (8/8) for O26, 100.0% (8/8) for O45, 100.0% (8/8) for O103, 37.5% (3/8) for O111, 87.5% (7/8) for O121, 75.0% (6/8) for O145 and 100.0% (8/8) for O157. Pen-level prevalence (at least one sample testing positive per pen) estimates were 74.0% (77/104) for O26, 73.1% (76/104) for O45, 99.0% (103/104) for O103, 4.8% (5/104) for O111, 37.5% (39/104) for O121, 19.2% (20/104) for O145 and 64.4% (67/104) for O157. These descriptive results provide insight on the feedlot- and pen-level fecal prevalence of *E. coli* serogroups in pre-harvest commercial feedlot cattle, yet determining the subset of isolates that are STEC (i.e., carry major virulence genes) is a critical next step.

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Prevalence of targeted enterohemorrhagic *Escherichia coli* in culled dairy cows

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Enterohemorrhagic *E. coli* (EHEC) serogroups O26, O45, O103, O111, O121, O145, and O157 (EHEC-7) account for the majority of cases of human illness due to EHEC in the U.S. and have been declared adulterants in non-intact, raw beef by the USDA-FSIS. The objective of this study was to determine the prevalence of EHEC-7 in fecal, hide, and carcass surface samples from culled dairy cows at harvest. One hundred culled dairy cows from the western U.S. were sampled over 5 weeks at a commercial abattoir (June to July 2014). Fecal, sponged hide and sponged carcass samples were enriched in *E. coli* (EC) broth and aliquots were subjected to immunomagnetic separation (IMS) treatments. Recovered IMS beads were spread plated onto STEC heart infusion washed blood agar with mitomycin-C, CHROMagar<sup>TM</sup> O157 with potassium tellurite, cefsulodin, and cefixime and CHROMagar<sup>TM</sup> STEC. Suspect colonies were screened as pools for Shiga toxin (*stx*) by PCR. Individual colonies from positive pools were tested for *stx1*, *stx2*, EHEC-7 O-groups, intimin (*eae*), and enterohemolysin (*ehxA*) by multiplex PCR. An aliquot of the



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enriched EC broth was also tested for EHEC-7 by mass spectrometry-based NeoSEEK™ (Neogen® Corp.) analysis, and by the Atlas® EG2 Combo assay (Roka® Bioscience) that identifies O157:H7 and non-O157 EHEC. EHEC-7 were recovered from 5.0% of fecal samples, 8.0% of hide samples and 1.0% of carcass samples by culture with a majority being O157:H7. By NeoSEEK™ STEC analysis, the prevalence of EHEC-7 was 26.0%, 65.0%, and 7.0% for fecal, hide and carcass samples, respectively. In descending order, a higher proportion of fecal and hide samples tested positive for EHEC O45, O145, O111 and O103, followed by EHEC O157, O26, and O121 by NeoSEEK™ STEC. For carcass samples, in descending order, a higher proportion tested positive for EHEC O103, O26, O145 and O157, followed by EHEC O45, O111, and O121 by NeoSEEK™ STEC. By Atlas® EG2 assays, 29.0%, 46.0%, and 28.0% were non-O157 EHEC positive and 29.0%, 51.0% and 3.0% were O157:H7 positive for fecal, hide and carcass samples, respectively. This data will be used to populate a microbial risk assessment model with the goal of reducing the occurrence and public health risks from EHEC-7 in beef.

048

Quantification of six non-O157 *E. coli* serogroups in cattle feces by spiral plating method

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**Purpose:**Six serogroups (O26, O45, O103, O111, O121 and O145) of Shiga toxin-producing *E. coli* (STEC), account for 70% of non-O157 STEC foodborne illnesses in humans (CDC report). Similar to O157, cattle are also reservoirs of non-O157 serogroups which harbor organisms in the hindgut and shed in their feces. Fecal contamination of food and water leads to human illnesses. With O157, a small subset of cattle, called “super shedders”, shed the organism at high concentrations ( $> 10^4$ ). It is not known whether non-O157 have a similar pattern of fecal shedding. Our objective was to evaluate spiral plating method to quantify the six non-O157 *E. coli* serogroups in cattle feces.

**Methods:** Initially, the spiral plating method was evaluated by spiking cattle fecal samples with ten-fold serial dilutions ( $10^{-1}$ - $10^{-8}$  CFU/ml) of pooled pure cultures of two different combinations (1) O26, O103 and O111; 2) O45, O121 and O145). Spiked fecal samples were diluted (1:100) in *E. coli* (EC) broth and 100  $\mu$ l of each dilution were spiral-plated onto a chromogenic medium. Concentration (CFU/g) was determined by counting the chromogenic colonies using counting grid. Ten randomly picked chromogenic colonies were tested individually by a multiplex PCR to confirm the serogroup. Additionally, cattle fecal samples (n=576), collected from a commercial feedlot in summer of 2013, were used to assess the applicability of spiral plating method to enumerate non-O157 *E. coli*. One-hundred microliters of 1:100 diluted feces in EC broth was spiral plated onto chromogenic medium. Concentration of each serogroup was determined based on the proportion of colonies (of the 10 chromogenic colonies tested) positive for the serogroup.

**Results:**The concentration of the serogroups by spiral plating was one log higher than the concentration used to spike. Of the 576 samples, 131 were quantifiable ( $>10^3$ ), of which 118 were O103, 19 O26, 19 O45, 2 O145 and 1 O121. Concentration of each serogroup was in the range of 3.0-6.5  $\log_{10}$  CFU/g of feces.

**Conclusions:**The spiral plating technique combined with PCR confirmation has the potential to be a useful technique to quantify non-O157 *E. coli* serogroups in cattle feces

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Modeling the intestinal concentrations of antimicrobials in animals

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The consequences of using antimicrobial drugs in farm animals include the potential for development of drug resistance not only in the targeted pathogens but also in by-standers such as enteric bacteria. While pharmacokinetics of antimicrobials related to the therapeutic effect is relatively well understood, the estimates of the intestinal drug concentrations are lacking. We considered which variables, beyond those used to approximate the therapeutic effect, would allow estimating the intestinal concentrations of antimicrobially-active drug or its metabolite. In particular, we considered the drug's intestinal passage time, degradation, and binding to the digesta. We used an animal model of a beef steer treated against a respiratory disease with chlortetracycline administered per oral or cephalosporin ceftiofur administered parenterally. The corresponding pharmacokinetic models were simulated keeping constant the values of the variables related to the expected therapeutic effect, while varying those related to the drug/metabolite intestinal fate. This modeling exercise demonstrated the extent to which the second group of variables may influence the selective pressure on the cattle enteric bacteria during antimicrobial treatment. The modeling approach taken is conceptually linked to the population pharmacokinetics of antimicrobials, and we discuss possible sources of inter- and intra-individual variability in the values of variables related to the intestinal fate of antimicrobials.

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Effect of heifer-raising practices on *E. coli* antimicrobial resistance and *Salmonella* prevalence among heifer fecal pats.

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Cattle movement and commingling have an important role in the inter-herd transmission of pathogens such as *Salmonella*. However, limited information is available on the effect of commingling on antimicrobial resistance and the fecal shedding of *Salmonella*. The objective of this study was to compare the resistance of *E. coli* and prevalence of *Salmonella* from fecal pats of heifers raised off-farm at multi-source heifer raisers (OFR) that raised heifers from at least 2 farms, and on-farm heifer raisers (ONR) with heifers from only that farm. A total of three OFR and three ONR were enrolled in the study and visited for sampling three times each. Half of the OFR samples were collected from pens with animals that had arrived at the farm within the previous 2 months (AR) and the other half from pens with animals that would be departing the heifer raiser in 2 to 3 months (DP). Age of cattle in pens sampled at OFR was used for sampling at ONR. On each farm visit an average of 24 pooled samples of three fecal pats were collected and placed in Para-paks. Samples were cultured and confirmed for *E. coli* and *Salmonella* growth using selective culture media. A total of 858 *E. coli* isolates (two *E. coli* isolates per pooled sample) were tested for susceptibility to 12

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antimicrobial agents using a Kirby-Bauer disk diffusion assay. *Salmonella* isolates were serogrouped. At the pen level, the odds of ampicillin resistance were 3.0 times greater among *E. coli* collected from OFR as compared to ONR. No significant difference between heifer raisers was observed for the remaining agents tested. Also at the pen level, *E. coli* from AR pens had significantly ( $P$ -value<0.05) higher odds of resistance to ampicillin, neomycin, streptomycin, and tetracycline as compared to DP pens. Prevalence of *Salmonella* was higher in ONR samples (15%;  $n=235$ ) as compared to OFR samples (4%;  $n=235$ ), however, this result was inflated by a higher prevalence of *Salmonella* at one ONR farm. Heifer-raising system did not have a major overall impact on selection of resistant *E. coli*, which was more strongly affected by the age of the animals sampled. Prevalence of *Salmonella* was relatively low on farms sampled, and no significant effect of heifer-raising system was apparent.

051

Using metagenomics to unlock the ecology of antimicrobial resistance in cattle production systems

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A complex ecology modulates the dynamics of antimicrobial resistance (AMR) within food production systems. Traditional microbiological methods provide only narrow glimpses into this ecology due to a reliance on phenotypic expression of resistance in cultured bacteria. Shotgun metagenomics can revolutionize research into food system AMR by providing access to all of the genetic material in samples, including all of the antimicrobial resistance determinants (ARDs). The goal of this study was to utilize a metagenomics approach to describe the AMR profiles ("resistomes") from diverse cattle production systems.

Environmental fecal, soil and water samples ( $N=34$ ) were collected from an organic and conventional dairy, a US and Canadian beef feedlot, and a ranch. Total DNA was extracted and shotgun sequenced on the Ion Proton. Sequence reads were aligned to a custom database of ARD sequences. Reads aligning to each ARD were summed and normalized by sample. Resistome diversity was compared across production and sample types. Associations between production type and ARD abundance were identified using zero-inflated Gaussian mixture models. Sequencing produced 1.05B reads (mean = 30.8M reads per sample, range 14.9M to 48.9M). Alignment identified 248 unique ARDs across all 34 samples. The number of unique ARDs within a sample ranged from 0 to 125. No ARDs were found in the water or soil collected from pasture ( $N=4$ ). ARDs conferring tetracycline resistance were most abundant, at 69% of all ARD-assigned reads (148,404/214,196). Diversity was significantly different between production and sample types, and significant differential abundance was detected for several ARD classes. This project represents the largest assemblage of agricultural metagenomic sequence data identified to-date, and provides proof-of-concept for use of metagenomics in AMR research. Initial results indicate that resistomes differ significantly by production type, and that pasture-based systems harbor significantly fewer ARDs than other cattle production systems. Overall findings highlight the unique insight that can be garnered from a metagenomics approach.

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Enterobacteriaceae producing extended spectrum beta-lactamases from wild birds on Ohio dairies.

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Objective: Extended-spectrum beta lactamases confer bacterial resistance to critically important antimicrobials. Livestock are an important reservoir for the zoonotic food-borne transmission of resistant enteric bacteria. Our aim is to describe the potential role of migratory and resident wild birds in the epidemiology of transmissible extended-spectrum beta lactamase-mediated bacterial resistance on dairy farms. Methods: Using mist nets, we captured and sampled 285 wild migratory and resident birds either immediately outside of or 600 feet away from freestall barns on three Ohio dairy farms. Individual swabs were used to obtain both a cloacal and external surface swab of feathers and feet from each bird. Samples were inoculated into MacConkey broth containing 2 ug/ml cefotaxime and inoculated onto MacConkey Agar with 8 ug/ml of cefoxitin, 4 ug/ml of cefepime, or 2 ug/ml of meropenem to identify the *bla*<sub>CMY</sub>, *bla*<sub>CTX-M</sub>, and carbapenemase phenotypes. Results: Fifty-eight birds (21.40%) produced cefoxitin-resistant isolates, representing the expected phenotype of *bla*<sub>CMY</sub>, and eight birds (3.0%) produced cefepime-resistant isolates, representing the expected phenotype of *bla*<sub>CTX-M</sub>, from either their cloacal swab/fecal sample or from their external feather/feet swab. There was no difference in the prevalence of either gene between migratory and resident birds or between the prevalence of *bla*<sub>CMY-2</sub> phenotype and distance from the barn. However, prevalence of the *bla*<sub>CTX-M</sub> phenotype was higher among birds sampled immediately outside the dairy barns compared to those sampled 600 feet away. Discussion: Our results suggest that wild birds can serve as mechanical and/or biological vectors for Enterobacteriaceae with resistance to extended spectrum beta-lactamases. Birds live in close contact with dairy cows and their feed, therefore transmission locally from farm to farm is possible. Finding a similar prevalence in non-migratory birds and those migrating from the Southern US, Central and South America, suggests the potential for regional and intercontinental movement of these genes via birds.

053

Prevalence and characteristics of *Salmonella* found on the paws and in the feces of free-ranging raccoons (*Procyon lotor*) in southern Ontario, Canada.

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Raccoons are common in urban and rural environments and can carry a wide range of bacterial agents, including *Salmonella*, that can affect human and domestic animal health. Although previous studies have reported that raccoons shed a variety of *Salmonella* serovars in their feces, it is unknown whether *Salmonella* can be carried and transmitted on the paws of raccoons. Our objective was to compare the prevalence of *Salmonella* on the paws and in the feces of raccoons in southwestern Ontario. Raccoons were sampled in a cross-sectional study from May to

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October 2012 where individuals were sampled repeatedly; 416 paired fecal and paw samples were collected from a total of 285 individuals. We detected *Salmonella* in 18% (75/416) (95% CI, 14-22%) and 27% (111/416) (95% CI, 22-31%) of paw and fecal samples, respectively. *Salmonella* was found both on the paws and in the feces in 10% (40/416) of raccoon captures, while 8% (35/416) had *Salmonella* on the paws but not in the feces and 17% (71/416) had *Salmonella* in the feces but not on the paws. A total of 10 *Salmonella* serovars were detected on paws and 14 in feces. *Salmonella* serovars Oranienburg, Newport, and Typhimurium were detected most commonly for both sample types. Antimicrobial resistant *Salmonella* was found in 3/416 fecal samples and 1/416 paw samples. The final multivariable model included the following explanatory variables: sex, sample type, season, and sex-season and sex-sample type interaction terms. In the final multivariable model, sex and interactions between sex and sample type and sex and season were significantly associated with the outcome. We noted significant differences ( $p < 0.05$ ) in the prevalence of *Salmonella* carriage between sexes that varied with sample-type and season. We suspect these differences in prevalence reflect sexual variation in dispersal patterns and foraging behavior across seasons that impact exposure to *Salmonella*. Because raccoons can carry *Salmonella* serovars known to infect humans and livestock on their paws and/or in their feces, they have the potential to mechanically and biologically disseminate *Salmonella* among livestock facilities and human recreational areas.

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Potential transfer of antimicrobial resistance (AMR) *Salmonella* and *Staphylococcus sciuri* after application of swine manure in the environment  
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The objective of this research is to study the transmission of *Salmonella* and *Staphylococcus* due to swine manure application in the environment at the multiple time points including day 0, 7, 14, and 21 representing swine farms in NC (n=2) and IA (n=7). A total of 990 soil samples (NC=205; IA=705) and 90 lagoon samples (NC=20; IA=70) were collected from swine farms and residential area. The isolates were characterized at the phenotypic and genotypic levels to compare them from different sources. Antimicrobial susceptibility (AST) for *Salmonella* was determined using Sensititre® with a panel of 15 antimicrobial drugs, while *Staphylococcus sciuri* was analyzed for resistance to a panel of 12 antibiotic drugs using Kirby-Bauer disk diffusion. PCR was used to identify the resistant determining genes. Genotypic characterization was done using pulse field gel electrophoresis (PFGE). Overall *Salmonella* and *S. sciuri* prevalence were 4.04% and 6.06% in soil and 44.44% and 21.11% in lagoon samples, respectively. We identified 7 serotypes including Anatum (27.5%), Altona (22.5%), Muenster (22.5%), Worthington (12.5%), Litchfield (7.5%), Mbandaka (5%), and Uganda (2.5%). Overall, we observed a decrease in *Salmonella* and *S. sciuri* prevalence overtime. A total of 29 (61.7%) of *Salmonella* isolates were MDR with the most frequent antibiotic resistance against sulfisoxazole (63.8%), tetracycline (61.7%), streptomycin (59.6%), and gentamicin (57.45%). All *S. sciuri* isolates were MDR with a high frequency of resistance to ampicillin, clindamycin, penicillin 95.5% and cefoxitin, ceftiofur 92.5%. In addition, all 67 of *S. sciuri* isolates carry *mecA* gene. According to preliminary PFGE result, we detected clonal relatedness based on geographic origin in both pathogens. The preliminary finding of our study highlight the potential role of manure in pathogen transmission in the environment, however, it is important to mention the relatively few positive samples we isolated in our study.

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Risk factors for antimicrobial resistance of *Escherichia coli* isolates from Ontario broiler chicken flocks at chick placement: A comparison of three production system types

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Antimicrobial use in broiler chickens has been previously identified as a risk factor in the development of antimicrobial resistant pathogens which can be transferred to humans. Our objective was to determine risk factors for resistance of *E. coli* isolates obtained at chick placement, with an emphasis on identifying differences between production system types. Seventy-four conventional, thirty-four antimicrobial-free, and seven organically-raised flocks distributed throughout Ontario, Canada were sampled throughout July 2010 to April 2012. For each flock, two pooled dust samples (feeders/drinkers, and floors/walls/fans) of the environment were collected immediately prior to chicks arriving from the hatchery, and six pooled swabs of meconium from chick pads representing a hatchery-level sample of 300 birds per flock were collected upon arrival. A questionnaire gathered information on hatchery and barn-level factors, including: antimicrobial use, vaccination, biosecurity, and management. Samples were submitted for isolation of *E. coli* using standard techniques, positive isolates were forwarded for susceptibility testing to 15 antimicrobials using an automated system. *E. coli* were frequently isolated from all production system types at both sampling times, regardless of sample type. There was no resistance of *E. coli* isolates obtained from chick pads samples to ciprofloxacin or from environmental samples to ciprofloxacin and azithromycin. Isolates originating from environmental samples collected from antimicrobial-free flocks had a decreased odds of resistance to trimethoprim-sulfa compared to those from conventionally-raised flocks ( $p = 0.031$ ), controlling for clustering at the flock and producer levels. Risk factors associated with resistance differed between sample type and antimicrobial agent. Ontario hatchery company from which chicks originated and the use of Infectious Bursal Disease vaccine at the hatchery were identified as risk factors associated with *E. coli* resistance to several antimicrobials. These findings suggest that practices occurring higher in the production chain may contribute to the selection of resistance to these antimicrobials.

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Predictors for contamination of informally traded ready-to-eat (RTE) chicken with generic (Biotype I) *Escherichia coli*

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Purpose: The study investigated the correlation between hygiene practices and contamination of RTE chicken sold by informal vendors with generic (Biotype I) *E. coli*.

Methods: A cross sectional study design using participatory research methods and laboratory isolation of generic (Biotype I) *E. coli* was adopted. Correlations between hygiene food handling practices and the likelihood of contamination of RTE chicken were assessed using univariable and multivariable logistic regression analysis. Results: Fifteen (6.32 %; CI95% = 3.91 - 10.47) of the samples tested were positive for *E. coli*. It was

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observed that the OR of contamination of RTE chicken at vending where flies were sighted was 6.42 (CI: 95% = 1.36 - 30.29; P = 0.02) times higher than when no flies were sighted. The odds of RTE chicken getting contaminated if the vendors intermittently washed their hands during serving of food was 11.47 (CI:95% = 2.60 - 50.49; P = 0.001) higher than if the vendors washed their hands between each serving. RTE chicken that was prepared from vending sites located more than 30 m from the vending site had an OR that was 6.14 (CI:95% = 0.91 - 41.27; P = 0.06) higher than RTE chicken prepared at sites located < 30 m from the vending site. If RTE chicken was held at a holding temperature below 70 °C before serving the OR of such chicken getting contaminated was 7.52 (CI95% = 0.83- 67.84; P = 0.07) higher temperatures.

Conclusions: The RTE chicken sold on markets surveyed had a low prevalence of contamination with organisms of enteric origin. The study identified presence of flies at the vending site, intermittent as opposed to washing hands between each serving, location of vending sites more than >30 m from the toilets and violation of holding temperatures as key predictors for contamination of RTE chicken. Informal vendors need to improve on their hygiene handling of RTE chicken to diminish the possibility of contamination of RTE chicken with *E. coli* Type I. In addition, vendors should be provided with proper ablation facilities within a reasonable distance from vending site to encourage hygiene to diminish risk of exogenous contamination of RTE chicken.

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Salmonella phenotypic and genotypic diversity in finishing swine

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Salmonella species are one of the major causes of foodborne diseases in the United States and worldwide. Molecular typing and quantitative methods are significant tools used to better understand the transmission and ecology of Salmonella, in order to implement pre-harvest control measures. The objectives of this study were to describe the Salmonella genotypes, serovar-specific concentration shed, distribution of the PFGE patterns (pulsotypes) of isolates from different ecological niches (i.e., barn environment, nursery and individual pigs) and evolution over time in a longitudinal study conducted in three finishing sites. Isolates from a total of 446 individual pig samples were serotyped and the samples were submitted for quantitative PCR. Thirteen *S. Derby*, one *S. Agona* and one *S. Johannesburg* isolate were from individual pig samples with a concentration greater than 103 /g feces. Among the 107 Salmonella isolates submitted for PFGE analysis, there were 29 distinct pulsotypes. PFGE genotyping results were consistent with the serotype findings. A significant heterogeneity of Salmonella pulsotypes (within the same serovar) was observed, and different combinations of pulsotypes were identified within and across sites and cohorts. These data indicate that each group of pigs introduced in the finishing barn established new pulsotypes in the barn. In addition, this study suggests persistence of the same pulsotype over several cohorts of pigs and potential residual-contamination within the barn between cohorts.

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Oral *Salmonella* challenge and subsequent uptake by the peripheral lymph nodes in calves

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Abstract Not Available

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High throughput environmental testing for *Salmonella* sp. using Matrix-Assisted Laser Desorption/Ionization-Time of Flight MALDI-TOF) technology

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Purpose: Animal-source exposures, including contact with animals, animal products, or their environments, are often implicated in outbreaks of non-typhoidal *Salmonella* sp. infections in humans. Livestock and poultry operations in particular are under increasing pressure to establish monitoring and control programs for pathogens such as Salmonella on their farms. While molecular detection methods are useful for screening large numbers of samples, they yield limited opportunities to gather additional epidemiologic information that can be obtained from live cultures. High-throughput testing with rapid identification of Salmonella isolates would enhance the feasibility of environmental monitoring. The purpose of the present study was to utilize selective enrichment culture methods and Matrix-Assisted Laser Desorption/Ionization-Time of Flight (MALDI-TOF) bacterial identification to perform large-scale environmental screening of food animal production facilities.

Methods: A total of 6786 samples from a variety of environments (manure handling areas, bedding, feed, water, and peripheral environment sites) were tested over a five month timeframe using selective enrichment in tetrathionate broth followed by culture on XLT-4 and brilliant green with novobiocin (BGN) agar plates. One to three suspect colonies were tested per sample.

Results: A total of 2189 positive samples were identified, and serogroups B, C<sub>1</sub>, C<sub>2</sub>, D<sub>1</sub>, and E Salmonella isolates were recovered. More than 5700 suspect colonies were screened from these environmental samples; up to 350 isolates were tested per day and identification to the serogroup could be completed within three days.

Conclusions: MALDI-TOF protein profiles proved highly discriminatory in differentiating *Salmonella* sp. from other environmental bacteria (*E. coli*, *Klebsiella* sp., *Leclercia* sp., *Enterobacter* sp., *Citrobacter* sp., *Pseudomonas* sp.) and provided a rapid, accurate method for Salmonella monitoring in these environmental surveys.

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Designing a risk communication strategy for health hazards posed by traditional slaughter of goats in Tshwane, South Africa

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**Purpose:** The present study assess the demographic profile of people from various taxi ranks who are involved in traditional slaughter of goats so as to determine the suitability of taxi ranks as a site for implementation of a risk communication strategy for mitigating risks associated with traditional slaughter of goats.

**Methods:** A cross sectional study design was employed to achieve the objectives of this study. A hybrid sampling design that involved both systematic random and purposive sampling was employed to select subjects for interviewing at the different taxi ranks. Structured and informal interviews conducted at taxi ranks were recorded and analysed using descriptive statistics and thematic analysis.

**Results:** One hundred and five (n=105) people were interviewed. This number included women (n=48) and men (n= 57). The median age of respondents was 40.6 and 44.3 years for women and men respectively. The majority of respondents (61.9%, n = 65) were from Gauteng Province. Sixty three (60%) respondents indicated that they had attained least secondary education, whilst 4.81% (n = 5) indicated that they had not received formal education. This study demonstrated that a cross-sectional study targeting people at taxi ranks gave access to people of all genders, various age groups and languages, and origin. It was observed that both men and women play an active role in traditional slaughter of goats.

**Conclusions:** In view of this, risk communication strategies should target women as well as men. It should be possible to use taxi ranks for successful dissemination of food safety and occupational health risk mitigation messages. Since the present study was limited to taxi ranks in Tshwane, the authors recommend that development of risk communication strategies aimed at mitigating risks associated with traditional goat slaughter should consider the dynamic nature of demographic and cultural norms.

061

Evidence from bioassays that commercial spray drying processes are effective at inactivating porcine epidemic diarrhea virus

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Porcine epidemic diarrhea virus (PEDV) emerged in the U.S. pig population in April 2013 and has quickly spread through most swine producing areas. Feed and particularly pig-based feed components such as spray-dried porcine plasma (SDPP) have been implicated in PEDV transmission. To determine the infectivity of PEDV RNA present in commercial SDPP, 40 3-week-old PEDV naïve pigs were randomly divided into five treatment groups. At day post inoculation (dpi) 0, NEG-CONTROL pigs were sham-inoculated, PEDV-CONTROL pigs received cell culture propagated PEDV, and SDPP-CONTROL pigs received a diet with 5% SDPP positive for PEDV RNA. PEDV-CONTROL pigs began shedding PEDV in feces by dpi 3 and seroconverted between dpi 7 and 14, whereas pigs in NEG-CONTROL and SDPP-CONTROL groups remained PEDV RNA and antibody negative for the study duration indicating no evidence of infectivity of the PEDV RNA in the SDPP lot utilized. To further determine the effect of spray-drying on PEDV infectivity, 14 3-week-old pigs were assigned to five treatment groups and were inoculated with raw porcine plasma spiked with PEDV (RAW-PEDV-CONTROL), porcine plasma spiked with PEDV and then spray-dried, raw plasma from PEDV infected pigs, spray-dried plasma from PEDV infected pigs, or spray-dried plasma from PEDV negative pigs. For the spray-drying process, a tabletop spray-dryer with industry-like settings was used. In the pigs infected with raw PEDV-spiked plasma, PEDV RNA was present in feces at dpi 3 and the pigs seroconverted by dpi 14. In contrast, PEDV RNA or antibodies were not detected in any of the pigs in the other groups. This provides evidence that the experimental spray-drying process used was effective in inactivating infectious PEDV. To evaluate a potential positive effect of anti-PEDV antibodies in SDPP on PEDV challenge, 4 days prior to PEDV challenge pigs were switched to and remained on a 5% SDPP diet through dpi 28 or were orally administered a commercial egg-derived liquid PEDV globulin product through dpi 6. Under the study conditions neither of these additions did significantly alter PEDV-shedding or overall disease course.

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Estimating the Number of Human Cases of Ceftiofur-Resistant *Salmonella enterica* serovar Heidelberg in Québec and Ontario, Canada (2003-2011)

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**Purpose:** To estimate the annual numbers of human cases of ceftiofur-resistant *Salmonella* Heidelberg in Québec and Ontario that are attributable to chicken consumption and prior antimicrobial use.

**Methods:** Annual provincial case estimates from Canadian surveillance (2003-2011) for *S. Heidelberg* were scaled to account for Canadian under-reporting and under-diagnosis estimates for non-typhoidal *Salmonella*. Annual proportions of ceftiofur-resistance in *S. Heidelberg* from Canadian surveillance and an etiologic fraction for chicken consumption were used to estimate annual provincial numbers of ceftiofur-resistant cases from eating chicken. Excess cases (ECs) were calculated by multiplying the etiologic fraction of cases attributable to prior antimicrobial consumption (EF<sub>AMU-R</sub>) for an unrelated reason, and infection with a ceftiofur-resistant strain of *S. Heidelberg*. The EF<sub>AMU-R</sub> was estimated using the odds ratio for prior antimicrobial use as a risk factor for infection with resistant, non-typhoidal *Salmonella* and the annual provincial prevalence of antimicrobial prescriptions. The model was developed on the framework of the US Food and Drug Administration, Centre for Veterinary Medicine's model titled "The human health impact of fluoroquinolone resistant *Campylobacter* attributed to the consumption of chicken." The stochastic model and sensitivity analysis were constructed and simulated using Palisade @RISK (v.6.0.0) in Excel 2010.

**Results:** The annual mean incidence of ceftiofur-resistant cases of *S. Heidelberg* (Québec/Ontario, cases/100,000 people) from chicken decreased from 8/7 in 2004 to 1/1 in 2007-QC/2008-ON, increasing to 7/5 in 2011. The mean ceftiofur-resistant ECs from chicken (Québec/Ontario, total cases) decreased from 71/123 in 2004 to 6/24 in 2007-QC/2008-ON, but increased to 62/91 in 2011.

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Conclusions: This model will support future work to determine the increased severity, mortality and health care costs for ceftiofur-resistant *Salmonella* Heidelberg infections. These results provide a basis for the evaluation of future public health interventions to address antimicrobial resistance.

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Extended-spectrum cephalosporin resistant nontyphoidal *Salmonella* recovered from clinical human infections in Ohio, USA.

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In the US, nontyphoidal *Salmonella* are a common foodborne zoonotic gastroenteritis pathogen. Invasive *Salmonella* infections caused by extended-spectrum cephalosporin resistant (ESCR) phenotypes are more likely to result in treatment failure and adverse health outcomes, especially in severe pediatric *Salmonella* infections where the extended-spectrum  $\beta$ -lactams are the therapy of choice.

To estimate the prevalence of ESCR *Salmonella* in clinical human isolates received between January, 2012 and June, 2014 at the Ohio Dept. of Health, we screened 3,175 cryopreserved isolates on Mueller-Hinton agar containing 2  $\mu$ g/ml cefotaxime. Of these, 70 *Salmonella* isolates (2.2%) expressed reduced susceptibility to 3rd generation cephalosporins. This subset was further screened on Mueller-Hinton agars containing 16  $\mu$ g/ml cefoxitin, 8  $\mu$ g/ml cefepime, and 2  $\mu$ g/ml meropenem to identify the *bla*<sub>CMY</sub>, *bla*<sub>CTX-M</sub>, and carbapenem resistance phenotypes, respectively. A majority of these isolates (n=59, 84%, 1.9% overall) expressed the *bla*<sub>CMY</sub> phenotype with an additional 6 isolates (8.6%, 0.2% overall) having the *bla*<sub>CTX-M</sub> phenotype, and 7 isolates exhibiting only cefotaxime reduced susceptibility. No *Salmonella* isolates were resistant to carbapenems. The 59 phenotypic *bla*<sub>CMY</sub> *Salmonella* isolates represented 15 serotypes, most commonly *S. Typhimurium* (n=14, 24%), *S. Newport* (n=9, 15%), and *S. Dublin* (n=7, 12%). The *bla*<sub>CTX-M</sub> phenotype included *S. Saintpaul* (n=3, 50%), *S. Agona* (n=1, 17%), and *S. Enteritidis* (n=1, 17%). Of the isolates expressing only reduced susceptibility to cefotaxime, the majority were *S. Enteritidis* (n=5, 72%).

Most *Salmonella* infections are the result of zoonotic foodborne transmission from a livestock reservoir where extended-spectrum cephalosporins are commonly used. Our observed prevalence of ESCR *Salmonella* causing clinical human illness (2.2%) was lower than NAHMS Surveillance ceftiofur resistant *Salmonella* statistics reported for livestock - - Swine, 2006 (14.6%), Dairy, 2007 (4.7%), and Cattle Feedlot, 2011 (7.7%).

064

Understanding the occurrence of *Escherichia coli* O157:H7 super-shedding infections in feedlot cattle

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The presence of *Escherichia coli* O157:H7 super-shedding cattle in feedlots have the potential to increase the overall number (bio-burden) of *E. coli* O157:H7 in the environment. It is important to develop strategies to reduce the bio-burden of *E. coli* O157 in feedlots by clarifying practices associated with the occurrence of super-shedders in feedlot cattle. The key to developing a management strategy is to (1) clarify the mechanism for why a small percentage of feedlot cattle become super-shedders and (2) determine if the ingested dose or a specific strain of *E. coli* O157:H7 result in a super-shedder infection.

To address this, (1) pen floor fecal samples and herd parameters were collected from four feedlots over a nine-month period, then (2) six strains of *E. coli* O157:H7, 3 strains isolated from normal shedder steers and 3 strains isolated from super-shedder steers, were inoculated into 30 one-year-old feedlot steers. Five steers were assigned to each *E. coli* O157:H7 strain group and inoculated with targeted numbers of 10<sup>2</sup>, 10<sup>4</sup>, 10<sup>6</sup>, 10<sup>8</sup>, and 10<sup>10</sup> CFU of bacteria respectively.

In the feedlots, prevalence of infection with *E. coli* O157:H7 for the 890 fecal samples collected was 22.4%, with individual pen prevalence ranging from 0%-90% and individual feedlot prevalence ranging from 8.4%-30.2%. Two samples had *E. coli* O157:H7 levels greater than 10<sup>4</sup> MPN/g feces, thereby meeting the definition of super-shedder. Light weight at entry to the feedlot and higher daily maximum temperature were associated with increased odds of a sample testing positive for *E. coli* O157:H7. In the experimental inoculation trial, the duration and concentration of shed *E. coli* O157:H7 suggests that the time post-inoculation and the dose of inoculated *E. coli* O157:H7 are important factors while the *E. coli* O157:H7 strain and shedding characteristic are not.

Under the conditions of this experiment, super-shedding appears to be the result of cattle ingesting a high dose of any strain of *E. coli* O157:H7.

So, strategies that prevent steers from exposure to fecal contamination are beneficial against the spread and transmission of super-shedding *E. coli* O157:H7 in feedlots.

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Effect of feeding preweaned dairy calves raw milk with residual concentrations of antimicrobials on the resistance of commensal fecal

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Approximately 33% of dairy farms in the United States feed preweaned calves non-saleable milk which can be contaminated with antimicrobial residues (waste milk). The objective of our study was to evaluate the effect of feeding preweaned calves milk containing antimicrobial drugs at concentrations below the minimum inhibitory concentration (sub-MICs) on selection of resistant fecal *E. coli* in calves from birth to weaning. At birth, thirty calves were randomly assigned to a controlled feeding trial where: 15 calves were fed raw milk without the addition of antimicrobial drugs (NDR), and 15 calves were fed raw milk with the addition of ceftiofur, penicillin, ampicillin and oxytetracycline at final concentrations in the milk of 0.1, 0.005, 0.01, and 0.3  $\mu$ g/ml (DRM). Fecal samples were rectally collected from each calf once a week starting at birth prior to the first feeding in the trial (pre-treatment) until 6 weeks of age. DRM calves had a significantly higher odds ratio (OR) for having a greater number of *E. coli* non-susceptible to ampicillin (OR 95% C.I.: 7 - 12), cefoxitin (OR 95% C.I.: 7 - 9), ceftiofur (OR 95% C.I.: 2 - 8), and tetracycline (OR 95% C.I.: 6 - 30). A significantly greater proportion of *E. coli* non-susceptible to ampicillin (week 3 to 6), cefoxitin (week 2 to 6), ceftiofur (week 2 and 6), and tetracycline (week 2 to 6) was observed in DRM calves when compared to NDR calves. *E. coli* isolates from the DRM group had an odds ratio of 13 (OR 95% C.I.: 8 - 20) for being resistant to 3 or more drugs when compared to the NDR group. These findings highlight the role that exposure of *E. coli* to sub-MICs of antimicrobials has on the increase in the proportion of antimicrobial resistance in preweaned calves.

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Longitudinal study of nasal carriage of *Staphylococcus aureus* in swine veterinarians and its implications for health

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In several countries, elevated MRSA prevalence in nasal swabs of livestock farmers and veterinarians is attributed to exposure to specific variants harbored by animals, but any associated health consequences remain poorly understood. We conducted a longitudinal study of *S. aureus* colonization and health events in a cohort of 66 US swine veterinarians. Subjects submitted nasal swab samples monthly for 18 months and data about exposure to pigs, and events of injury and infection. A subset (41) was also tested once to quantify *S. aureus* in swab samples. Monthly prevalence of *S. aureus* (58.3% to 82.4%) and MRSA (5.9% to 15.2%) exceeded US population estimates, and the predominant variants (MLST sequence type/spa type) detected were ST398/t034, ST5/t002 and ST9/t337 which similarly predominate among US pigs. Culture positivity was associated with recent contact with pigs. Based on detection patterns, veterinarians were classified into three groups: AP (41%), IP (58%) and NP (1%). Among AP subjects, 56% harbored a single sequence type over 18 months, and mean CFU per swab was higher than in the IP group. Subjects reported 130 skin or soft tissue injuries (0.11 per person-month), but no clinically significant infections. US swine veterinarians have substantial occupational exposure to animal variants of *S. aureus* and frequently experience superficial injuries without infection. Our data suggest that the substantial exposure to animal *S. aureus* variants among occupationally exposed individuals does not translate into substantial risk of clinical infection.

Abbreviation

MRSA Methicillin resistant *Staphylococcus aureus*

MSSA Methicillin susceptible *Staphylococcus aureus*

MLST Multilocus sequence type ST Sequence type

AP Always positive IP Intermittently positive NP Never positive

CFU Colony forming unit

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Bile salt hydrolase: a microbiome target for enhanced animal health

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Use of antibiotic growth promoters (AGP) has been associated with the emergence of antibiotic-resistant human pathogens of animal origins. The global trend of restricting the use of AGP in animal production necessitates the need to develop effective alternatives to maintain safety and productivity of food animals. Bile salt hydrolase (BSH) is an intestinal enzyme produced by diverse gut bacterial species and involved in host lipid metabolism. Our recent studies suggest that BSH inhibitors are promising alternatives to AGP for enhanced growth performance and animal health. Using a high-purity BSH from a chicken *Lactobacillus salivarius* strain, we have identified a panel of BSH inhibitors. However, it is still unknown if these inhibitors also effectively inhibit the function of the BSH from other bacterial species with significant sequence variation and substrate spectrum. In this study, we determined the inhibitory effect of identified BSH inhibitors on a BSH from a *L. acidophilus* strain. Compared to the BSH from *L. salivarius*, the *L. acidophilus* BSH showed significant sequence variation (35% aa identity) and different catalytic feature. Sequence alignment and structure modeling indicated the two BSH enzymes contain conserved, catalytically important amino residues and domain. His-tagged recombinant BSH from *L. acidophilus* was further purified and used in standard two-step BSH assay to test inhibitory effect of specific compound. All the identified BSH inhibitors also exhibited potent inhibitory effects on the *L. acidophilus* BSH. In conclusion, this study demonstrated that the BSH from *L. salivarius* is an ideal candidate for screening desired BSH inhibitors that may effectively inhibit intestinal BSH activity, a widely distributed function of the gut microbiota. Such BSH inhibitors are promising alternatives to AGPs for enhanced safety and sustainability of food animals.

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Investigating the efficacy of antimicrobial metaphylaxis in finishing pigs

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Mortality due to Porcine Respiratory Disease Complex (PRDC) in the late finishing period represents a significant economic loss for producers. Historically, it has been common practice to apply antimicrobial metaphylaxis (AM) to control the impact of PRDC. However, to date, there are no published studies exploring the potential benefits of these strategies. In this study, we investigated the value of AM in late finishing pigs endemically infected with the majority of the bacterial and viral agents that cause PRDC, but free of PRRSv. 732 pigs from four AIAO Wean to Market (WTM) lots (148-199 pigs per lot; 89 -100 days post-weaning), were enrolled in the study. In each lot all study pigs were housed in the same pen, identified, and divided into two cohorts based on starting body weight, sex, and rectal temperature. The cohorts received either control (C) or AM (Tulathromycin 2.5 mg/kg IM, Zoetis). Pigs were monitored daily and re-weighed on day 21. Post-treatment weight gain over the 21 day period was used to measure the value of AM. Data was analyzed using a mixed model with replicate as a fixed effect and co-variation for starting weight. No difference in weight gain was observed between AM and C pigs (17.1 kg vs. 16.7 kg,  $p=0.34$ ) in each individual lot, although there was a statistically significant difference in starting weight ( $p<0.001$ ) and weight gain ( $p<0.001$ ) between replicates. No interaction between replicates and treatment outcomes were detected. The AM treated pigs in the lowest starting weight quartile, showed an improved weight gain over controls (18.5 kg vs. 16.4 kg,  $p=0.005$ ) that was not present in any other weight quartiles. There was no interaction between treatment and replicate lot in the lowest weight quartile suggesting that the effect is consistent across sites, sources, and time. These results suggest that AM which is known to be effective against all the bacterial components of PRDC under routine commercial health conditions cannot be justified to improve animal performance. Weight may be a useful tool to target AM into subpopulations to minimize the use of antibiotics while improving economic outcomes and animal well-being through a reduction in disease.

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Role of direct and indirect transmission of different PRRSV genotypes within and between swine production systems in the US  
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Porcine reproductive and respiratory syndrome virus (PRRSV) causes what is still one of the swine diseases responsible for large economic losses in the US, despite of preventive and control measures implemented to reduce transmission within and between production systems. However, few studies have investigated how the different PRRSV genotypes are spreading among swine systems in the US and which are the most likely transmission pathways contributing to it. The aim of this study was to estimate the specific role of direct (e.g., animal movements) and indirect (e.g., airborne or local spread) transmission of PRRSV genotypes within and between different swine production systems in the US. A mixed Bayesian model was used to quantify the association between the pairwise genetic distance of two isolates belonging to the most frequent RFLP types (1-18-4, 1-18-2, 1-26-2, 1-4-4), the spatial and temporal proximity (i.e., pairwise spatial distance and pairwise absolute difference in time between isolates), and frequency and characteristics of swine movements.

Moreover, we developed a herd score index that summarizes the genetic diversity of PRRSV on site and that may provide the baseline framework for benchmarking and prioritizing interventions at an individual (i.e., high risk herds) or system level. All methods and results have been implemented in Disease BioPortal© (<http://bioportal.ucdavis.edu>), allowing the near real-time update of new isolates and of trade networks into the analysis, as well as, the user-friendly visualization of the results.

Results revealed significant differences in the transmission patterns for different RFLP types, which highlights the importance of implement system-specific preventive and control strategies based on their risk profile and their predominant PRRSV transmission pathways. Methods and results are intended to provide a better understanding of the spread of different PRRSV genotypes, allowing to quantify the specific role that animal movements and local spread may have in disease transmission in the swine industry and to identify high risk herds where surveillance and control strategies should be prioritized.

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Disease investigation using data from a PRRS area regional control and elimination (ARC&E) project in Ontario, Canada.

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Purpose: This study aimed to investigate spread of porcine reproductive and respiratory syndrome virus (PRRSV) in an area of Ontario (Canada). The primary objective was to investigate spatial location and truck network membership as risk factors for being positive for specific discrete PRRSV genotypes. The secondary objective was to estimate the most likely transmission pathway between herds within the area.

Methods: One hundred and thirty-six swine sites were enrolled in the project and information regarding location, production system, truck network and site PRRS status was collected. Sequencing of the ORF5 gene was conducted whenever possible for positive herds. Phylogenetic analysis was performed using MEGA6 and R v.3.0.2, and three km buffers were constructed using ArcMap10.1. Network analysis was conducted using Gephi and UCINET6. A trucking network component was defined as swine sites that were connected to at least one trucking company. Logistic regression models using the generalized estimating equation approach were constructed to investigate location and transportation as risk factors for being positive with PRRSV genotypes of interest. All models controlled for clustering of sites within production systems. In addition, the most likely transmission chain among swine sites was constructed in R 3.0.2 using nucleotide sequence data and sample submission dates.

Results: The phylogenetic tree included 46 sequences, and there were three evident "clusters" recognized (4% cut-off), which were used separately as outcomes for statistical models (so-called genotypes 1, 2 and 3). Twenty-one components were identified as part of the truck network. Spatial location was found to be a risk factor for being positive for one of the three genotypes (genotype 2;  $p = 0.035$ ), but not for the other two. There was a trend for truck membership to be a risk factor for being positive for genotypes 1 ( $p = 0.058$ ) and 3 ( $p = 0.068$ ). The transmission chain allowed for identification of sites that might be important targets for surveillance and disease control efforts.

Conclusions: In conclusion, the PRRS ARC&E database contains information that can be used for regional disease investigations.

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Behavioral aspects of oral fluid sample collection

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Objective: The use of oral fluid specimens in research and diagnostics has been the focus of recent investigations. The majority of this work has focused on the detection of pathogen-specific antibody or nucleic acid in the oral fluid matrix. The specific objective of the present study was to evaluate the effect of the number of ropes provided in the pen on oral fluid sampling.

Methods: 60 5-week-old pigs were divided into 2 groups of 30 in 2 pens of identical size and structure. Samples were collected at approximately 7:00 a.m every morning. The process of harvesting oral fluids and quantifying the sample is described in detail elsewhere (1). The effect of the number of ropes provided on oral fluid sampling was evaluated for 20 days. The number of ropes

(1, 2, 3, or 4) in the pen randomized across the 20 days, with each "rope treatment" repeated 5 times. Observations were taken at the group and individual level, with individual pigs chosen at random and marked with colored ear tags. To collect oral fluid, ropes were hung from bars at the corners of the pens. Four cameras synchronously took pictures to document pig behavior at 2 second intervals throughout the sampling using an external computer trigger. "Chewing" was defined as a picture showing a pig's mouth closed around the rope.

Results: Average sample volume for the group when 1, 2, 3, or 4 ropes were present was 29.9, 39.0, 57.3, and 79.2 ml, respectively. Participation among the group (avg. no. of pigs chewing a rope) when 1, 2, 3, or 4 ropes was 5.0, 7.0, 9.1, and 11.1 pigs per minute, respectively. Average participation (chewing on the rope  $\geq 1$  time) of the individual pigs at 1, 2, 3, or 4 ropes was 90%, 91%, 92%, and 97%, respectively.

Conclusions: The total volume of oral fluid, the number of pigs chewing rope(s), and the total time that pigs chewed rope(s) increased as more ropes were provided. The behavioral aspects of oral fluid collection have only been addressed in one publication (2), i.e., many questions remain



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to be answered. Regardless, these data imply that there is an ideal pigs:ropes ratio that provides for optimal diagnostic sensitivity. 1. Olsen C et al. 2013. J Vet Diagn Invest 25:328-335. 2. Seddon Y et al. 2012. Vet J. 193:180-184.

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Influenza H1N1 and H3N2 co-infection in pigs afterweaning.

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The objective of this study was to assess the dynamics of co-infection by H1N1 and H3N2 viruses in pigs after weaning. Individual nasal swabs were collected weekly from a cohort of 132 weaned pigs for 15 weeks. Swabs were tested for influenza A virus (IAV) matrix gene by RT-PCR and a set of 96 positive swabs was selected for complete genome sequencing. Two epidemic waves of IAV were identified at week 2 and 7 respectively. Twenty nine percent of the pigs tested positive more than once in non-consecutive weeks indicating that pigs became re-infected with influenza. Our intensive sampling using deep genome sequencing allowed us to identify three different IAV during the study period: two H1N1 viruses (1 gamma, 1 beta) and 1 H3N2 virus. We demonstrated that the prevalence of IAV in endemically infected populations changed significantly between weeks after weaning. In our study different IAV lineages co-circulated in pigs after weaning and the proportion of each lineage changed over time. The co-circulation of distinct IAVs, and the maintenance of some of the viral genotypes at low prevalence may have played a role in the maintenance of IAV in this wean to finish population. Acknowledgments: National Pork Board (NPB), Minnesota Super Computing Institute (MSI) and the BioMedical Genomic Center of the University of Minnesota (BMGC)

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Smartphone applications for veterinary data collection in Malaysia: feasibility and data usage in animal disease surveillance

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Animal health related data collection is one of the most significant tasks in animal disease surveillance. Reliable, timely and adequately structured data is imperative in order to ensure proper analysis and research, which will be useful in understanding outbreaks and better planning disease monitoring or surveillance efforts. Recent developments in smartphone technology have produced a novel communication stream, where users can now easily and quickly retrieve and send information via smartphone applications. The utilization of a single device with multiple utilities such as Global Positioning System (GPS), barcode scanner, and proximity sensors is convenient in obtaining valuable veterinary data that can be used in surveillance and disease control measures. The study presented here outlines the animal disease surveillance scenario that exists in Malaysia, specifically within the state of Johor; and designs a framework for prioritizing the utilization of smartphone application in obtaining livestock information in the country. For such purpose, first, we collect information from several local veterinary data streams (regional and local veterinary services, reference laboratories, etc.) and conducted a first risk factor analysis and risk mapping for bovine brucellosis using a multilevel logistic model. Then, a questionnaire was designed and integrated into the smartphone application to collect the necessary information in order to fill the identified data gaps, particularly in areas identified to be at high risk for disease occurrence. Once collected the new data, the multilevel logistic model is expected to be re-run including the new information to create updated risk maps. Final risk maps with and without incorporating information captured with smartphones will be compared and evaluated. Methods and results are expected to guide the design of surveillance and target interventions for more cost-effective preventing and control of animal diseases in Malaysia.

074

Characterization of the network of live fish movements in the Irish salmon farming industry and implications for disease prevention and control

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Methods: Records of domestic live fish movements were provided by the Irish Marine Institute, including date, origin and destination sites, spatial coordinates, life stage, species and quantity moved. Using social network analysis methods, we estimated network metrics, such as density, transitivity, and assortativity coefficient. Also, farm level indegree, outdegree, incloseness, and outcloseness were computed. Spatial clustering for farm level metrics was assessed using a scan statistic. The presence of trading communities was evaluated using the Walktrap community finding algorithm.

Results: There were a total of 130 salmonid fish movements, roughly 17.2 M fish, with 62 sites participating. Fish shipments varied in distance, depending on species and farm type, with trout sites trading locally, and salmon sites trading with both nearby and far away sites. Most of fish were moved in February-April, June-September and November periods. Network density and transitivity were low, 0.03 and 0.22, respectively. Assortativity for indegree was -0.14, a weakly negative correlation between connecting nodes' indegree, while for outdegree was 0.19, a weakly positive correlation. Assortativity for indegree and outdegree was 0.06. The spatial scan statistic detected significant clusters ( $p < 0.05$ ) only for incloseness and outcloseness in Galway and Donegal, respectively. There were a total of 8 cohesive subgroups detected by the random walk algorithm, of which 4 were large subgroups, comprised of between 8 and 16 sites each and that included at least one site that had a high node centrality measure.

Conclusions: Trade of live fish varied greatly in both space and time, with periods of higher activity and hot spots with higher centrality measures. The isolation of communities by elimination of links between them will be valuable for compartmentalization and implementation of disease control strategies. This is more relevant considering that communities gravitated around sites with high centrality measures such as outdegree, which have the potential to be "superspreaders" for disease outbreaks.

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A qualitative risk model for animal welfare concerns due to movement restrictions during a classical swine fever (CSF) outbreak

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**Purpose:** Animal welfare concerns, such as overcrowding and feed shortage, can quickly result from movement restrictions imposed during a disease outbreak. The objective of this study was to build a qualitative risk model to display the relationship between animal welfare concerns and different movement restriction strategies during a CSF outbreak.

**Methods:** Four different movement restriction strategies were evaluated in this study: (a) movement within premise, (b) movement between premises, (c) movement to slaughter houses, and (d) complete movement restriction. Overcrowding, feed shortage, and waste accumulation were the major animal welfare concerns considered initially. The identification of model parameters was done through literature search, analysis of Indiana premise identification data (USAHerds) as well as a roundtable discussion among experts in epidemiology, veterinary medicine, swine production, and animal welfare.

**Results:** The conclusions from the roundtable discussion were: (a) movement restricted to within premise is not a viable option in modern swine operations and (b) feed shortage and overcrowding are the main animal welfare concerns to swine herds subject to movement restrictions. The final qualitative risk model consisted of two parts: (a) movement restriction strategies and consequent animal welfare concerns (MR-AW) and (b) disease transmission (DT). Important parameters identified were time to animal welfare concerns, length of epidemic, age of pigs, proportion of pigs to be moved, and proportion of pigs to be killed due to welfare concerns.

**Conclusions:** This established qualitative risk model can be used as a template for the quantitative risk model, and can assist in decision making during a CSF outbreak.

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The Alberta Veterinary Surveillance Network: Creating a tool to track cattle diseases and movements in Alberta, Canada

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**Purpose:** The Veterinary Practice Surveillance (VPS)-Cattle project is one piece of the Alberta Veterinary Surveillance Network that collects daily information from private veterinarians about cattle diseases, non-disease routine procedures and farm and cattle demographics in Alberta. The objective of this project was to develop a software tool to visualize cattle disease events relative to cattle movements in the province of Alberta.

**Methods:** Records in the VPS Cattle dataset consist of: date of visit, county name, operation (cow-calf, feedlot, dairy or backgrounder), age of animal, submission type (disease or non-disease), syndrome and presumptive clinical diagnosis. Records in the movement dataset from the Alberta Livestock Identification Services (LIS) contain: movement date, cattle type (calf, cow, bull, etc.), number of animals, origin, destination, and inspection site of the movements as six-digit postal codes. The syndromic disease and non-disease data for VPS Cattle for 2011-2012 at the county level were linked with the movement data from LIS for the same time frame using a Postal Code Conversion File from Statistics Canada. A time series of the daily events with incoming/outgoing movements was constructed in Matlab. This allowed for further analysis of the relationships between movement and disease events.

**Results:** An interface was developed to segregate the VPS and LIS data by their respective attributes. It provided the ability to visualize animal movement by different data subsets from the VPS syndromic data as well as the ability to look at signals of movements versus disease and non-disease detections.

**Conclusions:** The Matlab interface offered the flexibility to work with data from disparate sources and to develop a prototype that can link, visualize and look for disease outbreak signals related to cattle movements in Alberta. This will allow for future work to link this tool with statistical methods to evaluate time series for event detection using KNIME (Konstanz Information Miner) platform to increase the speed of processing of data with a wide range of statistical techniques and workflows.

077

Using scan statistics to explore the relative performance of dead birds and mosquito pools in surveillance for West Nile virus in Ontario, 2002-2008.

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Surveillance of West Nile virus (WNV) in Ontario has consisted of enhanced passive surveillance of dead birds and active surveillance of mosquito-pools, but there is a need to evaluate these strategies retrospectively for their timeliness and predictive ability in relation to human cases. The objectives of this study were to compare the distribution and timeliness of clusters of WNV-positive dead birds and mosquito-pools in relation to human cases.

Data about the location and time of detection of WNV-positive dead wild corvids, mosquito pools and human cases in Ontario over the period 2002-2008 were explored using spatial scan statistics. Spatial scan statistics based on the exponential model were employed for spatiotemporal cluster detection using survival data about the time-to-first-positive dead bird, mosquito pool and human case within public health units (PHUs) across Ontario.

Statistically significant ( $p < 0.05$ ) space-time clusters of PHUs with short time-to-detection of first WNV cases were found using all data streams. Most clusters were located in the southern regions of the province but the dead bird clusters showed a more northern distribution during later years. There was geographic overlap between clusters of positive dead birds, mosquito pools and human cases during all years during which significant clusters were found. The dead bird dataset generally outperformed the mosquito pool dataset with respect to time of onset of clusters. In 2002, the most likely cluster of first-positive mosquito pools contained the most likely cluster of first positive human cases, and was detected one month earlier. In 2004, the most likely first-positive clusters among mosquito pool and human cases were identical in location, with the beginning of the mosquito pool cluster preceding the start of the human case cluster by 2 weeks. In 2008, the most likely human case cluster was

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contained within the dead bird cluster, which started 1 month before the onset of the human case cluster.

Information regarding the location and time-to-first-positive dead bird and mosquito pool within a health unit area show utility in surveillance for West Nile virus in Ontario.

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A meta-analysis of the effects of feeding active dry yeast of *Saccharomyces cerevisiae*, on milk production of lactating dairy cows

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**Purpose:** The purpose of this study was to use meta-analytic methods on previously published randomized control trials (RCT) to estimate the effect of commercially available active dry yeast products on milk production and other production measures in lactating dairy cows

**Methods:** Four hundred ninety seven published research articles were initially identified through an electronic literature search using 5 computerized search engines. Twenty-two papers with 25 comparisons met the final criteria for inclusion in the meta-analysis. These studies evaluated active dry yeast products from 7 different companies and were conducted in 13 different countries. Statistical analysis was conducted on the extracted production data using Comprehensive Meta-Analysis version 2.2.050 and STATA V. 12.1 using the metan routine.

**Results:** A random-effects meta-analysis showed estimated mean differences between cattle supplemented or not supplemented with active dry yeast were 0.81 kg/d (95% CI = 0.19 to 1.46), 1.04 kg/d (95% CI = 0.47 to 1.61), and 1.00 kg/d (95% CI = 0.40 to 1.59) for milk yield, 3.5% fat corrected milk and energy corrected milk, respectively. Mean differences in milk fat yield and milk protein yield were 0.05 kg/d (95% CI = 0.02 to 0.07) and 0.02 kg/d (95% CI = -0.01 to 0.05). Estimated mean difference for dry matter intake for active dry yeast was 0.00 kg/d (95% CI = -0.86 to 0.85,  $I^2 = 59.66$ ). There was high heterogeneity ( $I^2 = 48.40$ ) in the study outcome for milk yield. One sub-group analysis identified an area of heterogeneity to be where the study was conducted (in North America,  $I^2 = 0.0$  and outside North America,  $I^2 = 59.5$ ). Milk yield for 7 studies conducted in North American were 0.49 kg/d (95% CI = -0.45 to 1.43) versus 0.96 kg/d (95% CI = 0.10 to 1.83) for 13 studies conducted outside North America. Although the difference was not statistically different ( $P = 0.524$ ).

**Conclusions:** Active dry yeast supplementation failed to show a significant increase in milk production in studies conducted in North America (0.49 kg/d,  $P = 0.307$ ) or in ECM (0.54 kg/d,  $P = 0.260$ ).

079

Dairy cattle management factors that influence on-farm density of European starlings (*Sturnus vulgaris*) in Ohio, 2007 - 2009

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European starlings (*Sturnus vulgaris*), one of the most abundant wild bird species in North America, can contaminate livestock feed with their excreta potentially disseminating animal and zoonotic pathogens to dairy cattle. Consequently, identifying dairy farm management and environmental factors that attract European starlings to dairy farms has potential animal and public health implications. In this study, the number of starlings from barns, feed storage and manure storage areas was recorded from 150 dairy farms in summer and fall 2007 - 2009. Risk factors from a questionnaire administered during these two farm visits were assessed for possible association with the number of starlings per milking cow (starling density). Zero-inflated negative binomial models were fitted using these data. Year of visit, feeding method (i.e. whether the feeding was on an aisle, bunk or other) and feeding site (i.e. whether cows were fed indoors or outdoors), feeding method and site, manure removal frequency, and distance from the closest roost site were significantly associated with starling density. Starling density was significantly higher on farms visited in 2007 compared to those visited in 2008 and 2009. A significant interaction effect between feeding method and feeding site was identified. The effect of different feeding methods varied depending on whether they were used indoors or outdoors, but generally feeding outdoors increased starling density on the farm. The likelihood of zero starling counts in farms that removed manure from barns weekly or less frequently was higher compared to those that removed manure daily or after every milking. The odds of a zero starling count decreased with increasing distance of a farm from the closest night roost. Identifying risk factors that expose farms to starlings would assist in developing non-lethal strategies that minimize the number of birds and reduce the potential transmission of cattle and zoonotic pathogens.

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Spatial clustering of bovine tuberculosis outbreaks in Uruguay.

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Bovine tuberculosis (BTb) is a chronic disease of cattle caused by the infection with the *Mycobacterium bovis*. BTb prevalence in Uruguay has been traditionally low as a consequence of an active surveillance program in slaughterhouses and dairy farms. Between 2011 and 2013, however, the incidence of the BTb had increased, concerning farmers, industry, veterinarians and the government authorities.

The goal of this study was to assess the spatial dynamics of the BTb outbreaks reported in Uruguay between 2011 and 2013.

Data provided by the Ministry of Livestock Agriculture and Fisheries in Uruguay included information on the location (latitude, longitude), status (positive, negative), and, if applicable, date of detection of the outbreak, for dairy farms in the country. Fifteen, 26 and 16 outbreaks were reported in 2011, 2012, and 2013, respectively. The spatial distribution of the incident (new cases) and prevalent (cumulative active cases) outbreaks was analyzed using the spatial scan statistic and the Cuzick Edwards test, implemented in the SatScan and Cluster Seer software, respectively.

Results suggested a significant ( $p=0.05$ ) clustering in two of the three years, located in northwestern and southeastern Uruguay. Absence of spatial clustering in 2011 suggests an initial random dispersed distribution of outbreaks, with a posterior transmission and local dissemination, explained by the 2012 observed clusters. Control measures applied during the epidemic are reflected in 2013 outbreaks distribution, which shows a residual aggregation in the north-west area.

In summary, while BTb has been described in Uruguay since 1897 and has proven difficult to eradicate, spatial characterization of recent outbreaks may contribute to improvement of the effectiveness of the BTb control program in Uruguay through understanding of disease transmission in these outbreaks.

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The effect of morbidity on weaning weight of beef calves

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**Purpose:** Weaning weight is an economically important outcome to beef cow-calf operations. A number of factors are known to affect weaning weight including weaning age, gender, and age of the dam. Few studies have measured the effect of disease on weaning weight. The objective of this study was to quantify the effects of pre-weaning illnesses on the weaning weight of beef calves.

**Methods:** Birth to weaning health and performance data from 14,798 steer calves surviving to weaning over a 14 year period (1993-2006) at the US Meat Animal Research Center were analyzed. The effect of morbidity on weaning weight was tested using a generalized linear mixed model with a random effect of year. Calves were categorized to disease status by the age that disease occurred: no disease; early (0d to 60d); middle (61d to 120d); and late (121d until weaning). Statistical significance was set at  $\alpha \leq 0.05$ .

**Results:** The average age and weight at weaning was 185 (std dev = 26) days and 221 (std dev = 35) kg respectively. The average number of calves weaned per year was 1,057 (std dev = 228). Weaning weight increased by 0.76 kg for every one day increase in age at weaning. Over the 14 year period bovine respiratory disease (BRD) was recorded in 1,105 calves (7%), eye lesions in 615 (4%), and enteric disease in 251 (2%). Calves with BRD or eye lesions at any age prior to weaning were significantly lighter weight at weaning than calves without. Compared to calves without BRD, calves with early BRD were 25 kg lighter at weaning, and calves with BRD in the middle and late age periods were 8 kg lighter. Compared to calves without eye lesions, calves with eye lesions in early, middle, or late age periods were 28, 10, and 7 kg lighter at weaning, respectively. Calves with enteric disease in early or middle age periods were approximately 23 kg lighter weight at weaning than calves without enteric disease.

**Conclusions:** Both if and when calves experienced morbidity affected weaning weight. Morbidity in the first 60 days of life had the most detrimental effect on growth performance.

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Performance of FAMACHA scores for detecting anemia in sheep

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The FAMACHA scoring system is used to identify sheep or goats clinically affected by internal parasites. Although FAMACHA is commonly perceived to be highly correlated with anemia, the diagnostic performance of FAMACHA to predict anemia as measured by packed cell volume (PCV) has not been well characterized. The objective of this study was to evaluate the diagnostic performance of FAMACHA scores in sheep for identification of anemia.

From 2011 to 2014, 279 rams were scored using the FAMACHA system, and concurrent blood samples were taken to determine PCV. Anemia was defined as a PCV of <27%. The FAMACHA scores were evaluated for sensitivity, specificity, likelihood ratios (sensitivity/(1-specificity)), and predictive values.

No sheep in this study scored above 4. The majority of sheep classified as anemic at any FAMACHA cut-off value had PCV  $\geq 27\%$ . In this study, FAMACHA scores  $\leq 4$  did not differentiate anemic from non-anemic sheep. At a cut-off value of FAMACHA score 4, sensitivity to detect anemia was 0.70 (95% CI = 0.93, 0.35); specificity was 0.94 (95% CI = 0.97, .91). The likelihood ratio for a score of 4 was 12.6. At a cut-off value of FAMACHA score  $\geq 3$ , sensitivity was 0.80 (95% CI = 0.97, 0.44) and specificity was 0.74 (95% CI = 0.97, 0.91). The likelihood ratio for a score of 3 was 0.50. At a cut-off value of FAMACHA score  $\geq 2$ , sensitivity was 1.0 (95% CI = 1.0, 0.69); specificity was 0.23 (95% CI = 0.29, 0.18). The likelihood ratio for a score of 2 was 0.34. At a cut-off value of FAMACHA score  $\geq 1$ , sensitivity was 1.0 (95% CI = 1.0, 0.31) and specificity was 0.0 (95% CI = 0.0, 0.01). The likelihood ratio for a score of 1 was 0. Negative predictive value of scores of 3 or 4 exceeded 90% in populations with less than 20% prevalence of anemia. Positive predictive value of scores of 4, 3, and 2 exceeded 90% in populations with 50%, 80%, and 90% or greater prevalence of anemia, respectively. A score of 4 classified the greatest percentage of sheep correctly until anemia prevalence reached 0.7; then lower scores classified more sheep correctly.

A FAMACHA score of 4 had the greatest diagnostic usefulness. A FAMACHA score of 4 might enable modest sorting of anemic from non-anemic sheep except in flocks with high prevalence of anemia.

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Respiratory disease outbreak detection within a population of free-living chimpanzees (*Pan troglodytes schweinfurthii*)

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Infectious disease is a threat to the conservation of great ape populations, including the chimpanzee population of Gombe National Park. Due to a large proportion of disease-associated mortality among Gombe chimpanzees, a syndromic health surveillance system was established in 2004 to capture observational data of clinical signs associated with specific disease syndromes: respiratory, gastrointestinal, dermatologic, and wasting. Syndromic data collected through this system was utilized to describe the epidemiology of 5 major respiratory outbreaks recognized in the communities during the period of 2004-2012. There were no mortalities associated with the major outbreaks, although mean morbidity was 64% (SD  $\pm$  34%, range: 30-90%). The syndromic data was also used to establish baselines of disease frequency to more readily detect smaller respiratory disease outbreaks in the two habituated chimpanzee communities. Baseline levels of mean monthly counts and prevalences of respiratory disease were estimated and utilized to generate algorithms for outbreak detection. The baseline occurrence of respiratory disease, characterized by any combination of cough, sneeze, or rhinorrhea, was fairly low in both communities, with an average of less than one chimpanzee per month showing signs and a prevalence below 3%. Preliminary analyses reveal the occurrence of an additional 3 outbreaks in one community and additional 4 in the other, totaling 6 outbreaks in each community over the 9-year period. Syndromic surveillance analyses suggest that, despite previous assessment, respiratory disease is an infrequent occurrence among the habituated Gombe chimpanzees, but smaller respiratory outbreaks may be going undetected in real-time due to the low numbers of affected chimps during each event.

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(083 continued)

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084

The Symbiology of Epidemiologic Pursuits of Academia and Government.

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Symbiosis is the interaction process between two or more biological species but for purposes here will be extended to include the relationship between epidemiologists and preventive medicine scientists at

universities and those within government agricultural agencies. Which symbiotic relationship is most prevalent between these entities?

Mutualism? Commensalism? Amensalism? Competition? Which symbiotic relationship should exist? In Roger Pielke's, *The Honest Broker*, he establishes four idealized roles of science in policy and politics: the pure scientist, the science arbiter, the issue advocate and the honest broker of alternatives. Which role fits best for academia and for government? The stereotypical division of academic pursuits (alias Science), including educating students and conducting research, from the policy development and disease control interests of government is archaic. The aim of decision-making is to reduce uncertainty in a particular desired direction or to increase the likelihood of the desired outcome. The science of epidemiology matters critically in the processes of deciding between alternative courses of action for controlling or preventing animal disease. Epidemiology, through observational and experimental studies, helps us to understand the associations between disease and risk factors and subsequently between different choices for control and their likely outcomes. The recent emergence of novel swine enteric coronaviruses in the United States serves as an excellent model of appropriate and beneficial interactions resulting in good science, good policy and ultimately, decisions made towards control of an economically devastating disease. Specific examples of interactions and their results will be presented to illustrate the potential benefits of the ideal symbiotic relationship between universities and government agricultural agencies and the industries they serve.

085

Mannheimia haemolytica in feedlot cattle: associations with antimicrobial use, resistance and health outcomes

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Mannheimia haemolytica is a significant etiological agent in bovine respiratory disease in cattle. Objectives of this study were to explore risk factors for isolation of susceptible and resistant M. haemolytica in a commercial feedlot setting, and to explore associations between isolation and health outcomes.

Cattle (n=5,498) from 4 feedlots located in Alberta, Canada were randomly enrolled and sampled at arrival and later in the feeding period.

Samples were cultured for M. haemolytica and tested for resistance to 21 antimicrobials. Records of antimicrobial use (AMU) and health events were collected. Inferential analysis was conducted using multivariable GEE logistic regression.

Parenteral AMU rates were low, and resistance prevalence was <2% for most drugs. Parenteral drug administration within 7 days of sampling was associated with decreased likelihood of M. haemolytica isolation (OR 0.2, 95%CI =0.02 - 1.2, P=0.006), while parenteral AMU in penmates of enrolled cattle increased this likelihood (OR 1.5, 95%CI 1.05 - 2.2, P=0.02). Parenteral AMU was not associated with isolation of single-drug resistant M. haemolytica, but greatly increased the odds of recovering M. haemolytica resistant to ≥2 drugs (OR 23.9, 95%CI 8.4 - 68.3, P<0.0001). Cattle from which M. haemolytica was cultured on arrival were more likely to be diagnosed with fever within 10 days of arrival compared to culture-negative cattle (OR 1.7, 95%CI 1.1 - 2.4, P=0.07).

Contagious spread may underlie colonization and transmission dynamics, as AMU in pen mates of enrolled cattle increased the risk of isolating both susceptible and multiply-resistant M. haemolytica. AMU did not appear to be the primary driver of resistance in M. haemolytica, and AMU protocols that target high-risk and clinically ill cattle are likely efficacious.

086

Reporting guidelines for observational studies in veterinary medicine: STROBE-Vet

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Purpose: Details on the methods and results of observational studies are often not well reported in the veterinary literature. Our objective was to develop an extension of the STROBE (Strengthening the Reporting of Observational Studies in Epidemiology) statement for studies in veterinary medicine.

Methods: A consensus meeting of 17 experts was held in May 2014 to develop an extension of the STROBE statement that addressed observational studies in veterinary medicine with health, production, welfare, and food safety outcomes. To prepare for the meeting, an e-mailed survey was conducted to identify specific issues for discussion.

Results: To meet the needs of a STROBE statement for trials in veterinary epidemiology, the consensus was that 16 items on the 22-item STROBE checklist needed some modification, including additions to 10 items: item 3 (objectives), item 5 (setting), item 6 (participants), item 7 (variables), item 8 (data sources/management), item 10 (study size), item 12 (statistical methods), item 15 (outcome data), item 16 (main results), item 22 (transparency). The methods and processes used to create this extension were similar to those used for other STROBE extensions.

Conclusions: The use of this extension to the STROBE statement, which addresses issues unique to veterinary epidemiology, should improve the quality of design and reporting of observational studies with animal health, production, welfare and food safety outcomes.

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Mathematical disease transmission models for livestock populations: A scoping review

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The spread of infectious diseases within agricultural systems has become a global problem. Disease outbreaks in food animal populations have important social, environmental and economic costs, erode consumer confidence in food products and often present a risk to human health. Much research attention has focused on mathematical modeling approaches to describe the dynamics of pathogens within human populations. These models have contributed to our understanding of disease dynamics and how best to intervene to prevent the introduction and spread of human infectious diseases. For complex agricultural host-pathogen systems, the development and use of mathematical models to support decision-making has been more diffuse. The goal of this study was to identify and evaluate the use of mathematical disease transmission models within the field of veterinary epidemiology. We mapped the peer-reviewed literature describing livestock disease transmission models for equine, swine, poultry, and cattle populations published in the past 20 years. Our findings demonstrate an increase in the development and use of dynamic, mechanistic disease transmission models over time, with cattle historically being the most represented species. However, modelling research remains only a very small portion of the total research effort invested in advancing our understanding of livestock infectious diseases. Our survey of the field demonstrates that modelling research has been highly concentrated within specific host - pathogen combinations (e.g. cattle and foot and mouth disease). We have identified major knowledge gaps and suggest that dynamical models can play an important role for integrating empirical data, supporting veterinary health decisions, and guiding innovative research.

088

Does exercise-induced pulmonary hemorrhage affect career longevity and performance among South African Thoroughbred racehorses?

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Purpose: Exercise induced pulmonary hemorrhage (EIPH) has a high incidence among Thoroughbred racehorses, causing it to be a major concern in the racing industry. Most research efforts have been focused on evaluating pathophysiology and methods for prevention. Some studies have assessed the association of EIPH occurrence and concurrent racing performance, but few have investigated long-term impacts of EIPH on career performance. Two recent studies that evaluated long-term impacts of EIPH found no association with career longevity. Therefore, we sought to substantiate findings related to EIPH and career performance (longevity, races, wins, places, and earnings) among a population of South African Thoroughbred racehorses not treated with furosemide or nasal dilator strips.

Methods: Two populations of South African Thoroughbred racehorses were combined: a randomized, placebo-controlled, crossover field trial and a cross-sectional study evaluating EIPH at five racetracks. Both populations were followed until retirement. Combined, 1,034 horses underwent post-race tracheobronchoscopic examination, independently evaluated as severity grade 0-4 by three investigators blinded to the horses' identity and race performance. Racing records for all horses were obtained from a commercial database after retirement from competitive racing and were summarized into career performance variables and analyzed using Cox proportional hazards regression.

Results: Overall, 70% of horses showed evidence of EIPH. Controlling for age, sex, and original study population, horses with EIPH started in approximately 4 more races than horses without (95% CI 1.99 to 6.18). Lifetime earnings were significantly, but not meaningfully, higher among horses with EIPH grade > 1 compared with those with EIPH grade 0 (adjusted average change in earnings 1.7 Rand, 95% CI 1.1-2.6). EIPH was not significantly associated with career longevity (adjusted HR 0.9, 95% CI 0.8-1.01), lifetime wins, or lifetime places.

Conclusions: EIPH does not appear to be associated with career performance.

089

Human Q fever: seroprevalence and exploration of risk factors for *Coxiella burnetii* exposure in small ruminant farm workers and veterinarians/veterinary students

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*Coxiella burnetii* is a zoonotic bacterium that causes Q fever in humans; sheep and goats are considered important reservoirs. Symptomatic acute disease manifests primarily with self-limited febrile illness, severe headache, atypical pneumonia, or granulomatous hepatitis; endocarditis is the most common presentation of chronic Q fever.

We conducted two cross-sectional studies to investigate the seroprevalence and risk factors for *C. burnetii* exposure in small ruminant farm workers and veterinarians/veterinary students in Ontario.

Data collection included blood sampling and completion of questionnaires related to demographics, lifestyle factors, on-farm practices, and medical history. Sera were analysed at the Public Health Ontario Laboratory with an immunofluorescence assay (Focus Diagnostics); phase I or II IgG titres  $\geq 1:16$  indicated seropositivity.

For the farm worker study, 172 people ( $\geq 14$  years of age) from 78 farms participated between August 2010 and March 2012. Individual- and farm-level seroprevalence (where  $\geq 1$  person tested positive) for *C. burnetii* were 64.5% (111/172, 95% CI=57.2-71.4) and 76.3% (58/76, 95% CI=65.8-84.6), respectively. Increasing proportions of seropositive sheep/goats on-farm, and working on dairy goat farms (compared to dairy sheep and meat goat farms) were positively associated with farm worker seropositivity in a mixed logistic regression model that accounted for clustering by farm. A history of ever having smoked tobacco was also marginally significant.

For the veterinary study, data were collected from 32 veterinarians/veterinary students who attended a Small Ruminant Veterinarians of Ontario meeting in February 2014; 59% (19/32, 95% CI=41.9-75.2%) were seropositive. Practicing veterinarians (compared to students) and being aged >30 years (compared to 18-29 years) were associated with higher odds of seropositivity in univariable exact logistic regression models.

Exposure to *C. burnetii* was common among the small ruminant farm workers and veterinarians/veterinary students studied. These results can help support the development of prevention and control guidelines to reduce risk of exposure in these populations.

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Rare disease epidemiology: results from the national scrapie prevalence study

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**Purpose:** Scrapie is a rare but fatal and reportable disease in goat and sheep in Canada. The prevalence of scrapie is believed to be 1 case per 2000 animals or lower. Some countries estimate their prevalence at a level below 0.01%. A national prevalence estimation study was conducted 2010 to 2012, to inform a future scrapie eradication program for Canada.

**Methods:** Sampling of the seemingly healthy small ruminant population was conducted at abattoirs. The sample size target for sheep was 15,000 animals. For goats no sample size was estimated and goats were sampled when available at randomized sampling days and locations for sheep.

Sampled sheep were traced back to their farm of origin, while goats were analyzed under the assumption that the province of the sampling location is the province of origin.

For rare diseases advanced statistical methods are required to estimate the period prevalence and respective confidence intervals. Confidence interval estimation for rare event prevalence estimates is performed using the Wilson and Agresti-Coull estimates with and without stratification by province.

**Results:** A total of 7 scrapie cases were identified and confirmed using two independent diagnostic tests.

**Conclusions:** In conclusion, rare diseases epidemiology requires analytical methods that go beyond the generally applied methods and offer surprising results. The period prevalence of scrapie in Canada is lower than was presumed. Sample size recommendation for future surveillance and eradication are presented.

091

Epidemiology of *Salmonella* spp. among feral pigs in Texas

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While it is well established that livestock are a key reservoir for *Salmonella*, the importance of wildlife species as reservoirs for this zoonotic foodborne pathogen is poorly defined. The population of feral pigs (*Sus scrofa*) in the United States might be as high as 8 million, with an estimated 2.6 million in Texas alone. Feral pig activity can lead to fecal contamination of crops and surface waters, pathogen transmission to livestock, and direct transmission to humans through contact or pig meat consumption. Our aims were to estimate the prevalence of *Salmonella* among feral pigs in Texas and to characterize the isolates, thus facilitating an assessment of public health risk presented by this wildlife reservoir. We have an ongoing active surveillance program for *Salmonella* fecal shedding among feral pigs in Texas. This program is supported by a collaboration with USDA-APHIS-Wildlife Services, which has a dedicated system in place for managing feral pig damage and disease concerns throughout the state. Fecal samples are being sent to Texas A&M University for *Salmonella* isolation using standard bacteriologic culture techniques. Isolates are characterized through antimicrobial susceptibility testing and molecular determination of serovar. Date, geographic location, and data on each pig are collected. To date, fecal samples have been collected from 222 feral pigs in 35 counties throughout Texas. Ninety-one (41.0%) samples from 27 counties were culture-positive for *Salmonella* spp. The odds of being positive for *Salmonella* were marginally higher among juvenile pigs than adults (OR = 1.6, p = 0.1). Four percent (2/53) of the *Salmonella* isolates tested against a panel of 15 antimicrobial agents were resistant to at least one drug. Fifteen serovars were detected among 33 serotyped isolates; the most common serovars were Montevideo (n=4) and Newport (n=4). This ongoing study is one of the first to shed light on the ecology and epidemiology of *Salmonella* among feral pigs in the U.S. Our data indicate that *Salmonella* is frequently found among feral pigs in Texas, with important implications for public health.

092

Risk mapping of Avian influenza in California using Multiple-criteria decision analysis.

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The recent case of Avian Influenza (AI) in California (CA) (H5-LPAI, April 18, 2014) and the potential risk of H7N3 introduction into the US from ongoing infections in Mexico through migratory birds highlights the urgent need to develop and implement solutions to protect CA poultry operations (POs) against AI. Because some waterfowl and shorebirds can be natural reservoirs for AI viruses (AIV) and therefore are considered the primary source for disease transmission into poultry, mapping the occurrence of AI in wild birds will allow the identification of high-risk areas for poultry exposure to AI virus. Similarly, there is a need to integrate the environmental, climatic and anthropogenic factors associated with AI outbreaks in POs. The study here integrates space-time information regarding AI surveillance and demographics of wild birds and poultry, environmental, climatic, agricultural, socio-cultural and economic risk factors using multiple-criteria decision analysis (MCDA) to produce high resolution (cell size=50m2) high-risk maps for AI occurrence in CA poultry industry. First, a systematic review was conducted to determine the magnitude of the association between hypothesized factors and AI occurrence. Second, spatial data on the identified factors were collected and isopleth maps were created for each of them (i.e., layers). Third, all layers were integrated using weighted linear combination implemented in ArcGIS 10.2 for the generation of the final risk map for AI occurrence in CA. Finally, an extensive sensitivity analysis was conducted to quantify the impact that changes in layer weights have on model outcomes. Results reveal that the risk of AI occurrence in California is heterogeneously distributed, with high-risk areas mostly clustered in specific areas of the South-West and North-West of CA. These findings will inform producers about the potential risk of AI exposure and the need to increase biosecurity into their operations. Furthermore, they will provide the foundations for targeting interventions and implementing risk-based surveillance strategies to better prevent and control AI in CA poultry industry.

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Within-farm spread of highly pathogenic avian influenza in Korean outbreaks of H5N1 and H5N8 virus types

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**Purposes:** To understand the potential risk of spread for different virus types of avian influenza, patterns of bird-to-bird transmission were explored for five epidemics of highly pathogenic avian influenza occurred in Korea. Characteristics for each of previous four epidemics (2003/2004, 2006/2007, 2008, and 2010/2011) H5N1 and the latest (2014) H5N8 types were compared.

**Materials:** A stage-changing S(susceptible)-L(latently infected)-I(infectious)-R(removed) model was used to describe the specific pattern of within-farm spread in chicken and duck flock, respectively. In chicken farms, the 'R' stage meant 'dead', while it was 'antibody-formed' or 'dead' in ducks. Input values of parameters were determined based on pathogenicity tests using strain of virus isolated in each of five epidemics of HPAI.

**Results :** In a chicken farm raising 20,000 individuals, it took 20 days to infect 10% of population, while it took less 15 days in case of H5N1 type virus. For duck farms, 10% of prevalence arrived in 19 days with H5N8, while it took 12 days H5N1.

**Conclusions :** Spread of HPAI virus was slower in the 2014 epidemic of H5N8 than previous epidemics of H5N1. Meanwhile, spread of HPAI seemed faster in chicken than duck flocks. This phenomenon is to some extent due to the expression of clinical signs including deaths.

## Immunology

095

Evaluation of porcine epidemic diarrhea virus transmission and the immune response in growing pigs

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Clinical disease during a porcine epidemic diarrhea virus (PEDV) outbreak in a naïve swine breeding herd is well chronicled; however, endemic PEDV infection in a pig population is not well described. The aims of this study were to determine the duration of infectious PEDV shedding, evaluate PEDV-specific antibody response, and demonstrate protective immunity against a second PEDV exposure in nursery pigs. On day 0 (D0), a 4-week-old pig was challenged with PEDV and 14 naïve contacts were comingled. On D7, 9 contact pigs were moved to a new room to serve as the principal virus reservoir group (PG) and comingled with 1 naïve age-matched sentinel (S1). Three days later, the S1 pig was moved to a separate room until necropsy. This process was repeated on D14, 21 and 28 with pigs S2, S3 and S4. On D49, 5 naïve age-matched pigs (N) and the PG were challenged (N/C, PG/C) with homologous virus and euthanized on D78. Rectal swabs were collected daily and tested for PEDV RNA using RT-PCR. Serum samples were collected weekly and tested for PEDV antibody using an ELISA. PEDV RNA was detected in S1 and S2 within 1 day of contact, but not detected in S3 or S4. All PG pigs were PCR-positive from D3-11, with some intermittently positive to D42. All PG pigs were negative post-challenge (PC). All N/C pigs were positive by 3 days PC and 3/5 pigs until 13 days PC. Based on ELISA, 2/9, 8/9 and 9/9 PG seroconverted by D7, 14, and 21, respectively. S1 and S2 seroconverted by 7 days post-contact while S3 and S4 remained seronegative. By 7 days PC, 2/5 N/C seroconverted; all were positive from 14-28 days PC. Evaluation of PEDV-specific memory B and T cells is in progress. Mild disease was observed for one week in the PG post-contact with the inoculated pig. Sporadic mild diarrhea was observed in the N/C lasting for several days. The PG shed infectious virus out to 14-16 days based on infection of S1 and S2, however not enough infectious virus was shed to infect S3 or S4. Although the PG were PCR positive for several days post contact with S3 and S4, the virus detected may have been below the threshold of infectivity for age-matched pigs. Future studies will involve testing PCR positive rectal swabs collected after day 21 for infectious virus using bioassay techniques.

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In vitro evaluation of serological cross-reactivity and cross-neutralization between the U.S. PEDV original and variant strains

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Porcine epidemic diarrhea virus (PEDV) was detected in U.S. swine in April 2013. Initially it was thought there was only one genotype of PEDV circulating in U.S. swine (designated as U.S. PEDV original strain). However, in late January 2014, our group identified a distinct PEDV strain in U.S. swine (designated as U.S. PEDV variant strain) that is genetically different from the U.S. original strain. Little is known about the phenotypic features of this U.S. PEDV variant. The current immunofluorescence antibody (IFA) assays, ELISA and virus neutralization (VN) tests offered at veterinary diagnostic laboratories for detecting PEDV-specific antibody are based on the U.S. PEDV original strain. It remains to be determined if these assays can detect antibodies against the U.S. PEDV variant strains and vice versa.

Three groups of PEDV-negative, 3-week-old pigs (five pigs per group) were experimentally inoculated with a U.S. PEDV original strain isolate, a variant strain isolate, and culture medium (negative control), respectively and sera collected at 0, 7, 14, 21 and 28 days post inoculation were tested by the following serological assays: 1) PEDV IFA antibody assays using the original and variant strains as indicator viruses, respectively; 2) VN tests against the original and variant strain viruses, respectively; 3) PEDV whole virus (original strain) based ELISA; 4) PEDV original strain S1-based ELISA; and 5) PEDV variant strain S1-based ELISA. It was found that the antisera against the original strain reacted with both original and variant viruses, and the antisera against the variant strain also reacted with both original and variant viruses, as examined by IFA. The antisera against the original strain and the antisera against the variant strain neutralized both the original and variant viruses. Testing by various ELISAs is ongoing. The detailed IFA and VN titers and ELISA results will be presented.

Our data indicate that the antibodies against U.S. PEDV original and variant strains similarly cross-reacted and cross-neutralized the two strains *in vitro*. The current IFA, VN, and whole virus based ELISA assays are able to detect antibodies against both U.S. PEDV original and variant strains.



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Relationship between maternal immune status and neonatal protection against PEDV infection

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**Objectives:** The objective of this project was to determine the level and isotype of anti-PEDV antibody necessary to protect neonatal pigs against clinical PEDV infection. The experiment was conducted in two parts. Part 1 evaluated PEDV protection in piglets from sows previously infected with PEDV. Part 2 assessed PEDV protection in neonates with specific levels of PEDV antibody. **METHODS:** In Part 1, 2 negative control sows and 7 sows previously infected with PEDV ("principals") were acquired from commercial farms at ~100 days of gestation and housed under experimental conditions. Piglets derived from principals were orally inoculated with  $1 \times 10^3$  TCID<sub>50</sub> PEDV (USA/Iowa/18984/2013) at 2 days of age while piglets from control sows remained unchallenged. Thereafter, clinical signs and body weight were recorded daily through the termination of the experiment on day post inoculation (DPI) 12. Specimens for testing included feces and serum from piglets, colostrum and milk, serum and feces from sows. In Part 2, piglets from 7 PEDV-naïve sows were administered specific levels of anti-PEDV antibody (harvested from Part 1) and then inoculated with  $1 \times 10^3$  TCID<sub>50</sub> PEDV at 2 days of age. Observations and sampling were performed as in Part 1 through DPI 12. **Outcome and Impact:** In Part 1, neither clinical signs nor diarrhea were observed in control sow litters (n = 2); piglet mortality was 0 and 7.14%; and piglet average daily gain (ADG) was 0.19 and 0.2 kg/day. In litters from "principal" sows (n = 7), diarrhea was observed in 27.3 to 100% of piglets; mortality ranged from 0 to 40%; and piglet ADG ranged from 0.02 to 0.19 kg/day. Testing (antibody and PCR) and statistical analyses are in progress.

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Interaction of interferons and mTOR signaling underlying PRRSV infection

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Animal immune and metabolic systems interact to elicit effective immune responses during viral infections. The signaling pathway mediated by the mammalian target of rapamycin (mTOR) complex (mTORC) is key to cellular metabolism and implicated in various disease responses. Recent studies highlight the critical role of mTOR signaling in regulating heterosubtypic protection against viral infection. Little is known about how interferons coordinate immunometabolic involvement in antiviral responses. We have examined the involvement of mTOR signaling in infection by porcine reproductive and respiratory syndrome virus (PRRSV) and determined the components in mTOR signaling that were regulated by interferons and PRRSV infection. Suppression of mTOR signaling by PP242, a non-selective inhibitor for both mTORC1 and mTORC2, significantly inhibited PRRSV infection in Marc-145 cells (70-80%), alveolar macrophages (30-50%), and monocyte-derived dendritic cells (30-50%) cells. The PRRSV-suppressive effect of PP242 at 2.5  $\mu$ M was comparable to porcine IFN- $\alpha$ 1 and IFN- $\beta$  at 0.2 and 2 ng/ml, respectively. The mTOR activator, MHY1485, reversed the antiviral effect of PP242; however, rapamycin, a selective inhibitor for mTORC1, had little effect on PRRSV suppression. Further studies showed that expression and activation of several critical components of mTOR signaling, including protein kinase B (Akt), Raptor, Rictor, and P70S6K were co-regulated by both IFN treatment and PRRSV infection. For example, phosphorylation of Akt was stimulated by type I IFNs, but suppressed by PRRSV infection and, IFN- $\alpha$ 1 and IFN- $\beta$  exerted subtype-specific regulation of Akt phosphorylation. In summary, these findings support that mTOR signaling has a bi-directional loop with the type I IFN system and suggest that some components of mTOR signaling may serve as targets for studying immunometabolic regulation of IFNs and implicate for antiviral regulation. This work was supported in part by USDA AFRI NIFA/DHS 2010-39559-21860 and USDA NIFA 2013-67015-21236.

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Predicting vaccine efficacy for food animals using the Epitope Content Comparison (EpiCC) tool: Application to PRRSV

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**Purpose:** Predicting Vaccine Efficacy for Food Animals using the Epitope Content Comparison (EpiCC) Tool: Application to PRRSV

**Methods:** We previously developed a set of Swine Leukocyte Antigen (SLA)-restricted epitope prediction tools (PigMatrix). We further modified this tool to define relatedness based on T cell epitope content. Using EpiCC, we screened GP5 sequences from 8 PRRSV and 2 modified live virus (MLV) vaccines. Epitopes predicted to bind to common class I SLA alleles were identified and compared pairwise between strains for calculation of an epitope-based relatedness score (EpiCC score).

**Results:** A distance EpiCC score matrix was constructed and used to build an 'epi-phylogenetic tree' that depicts the relatedness between GP5 proteins based on epitope content.

**Conclusions:** EpiCC provides an objective approach to aid pork producers in vaccine selection when a PRRSV strain is introduced into a herd, and to select viral epitopes for incorporation into a MLV vaccine. Finally, EpiCC may also be used for analyses of evolutionary drift via epitope deletion and for bio-surveillance.

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Evaluation of cross-protection in Foster<sup>TM</sup> PRRSV vaccinated conventional swine challenged with a contemporary, heterologous lineage 9

PRRSV field isolate. **D. Magstadt**<sup>1</sup>, P. Gauger<sup>1</sup>, D. Madson<sup>1</sup>, E. Burrough<sup>1</sup>, P. Arruda<sup>1</sup>, K. Harmon<sup>1</sup>, A. Pillatzki<sup>2</sup>, Q. Chen<sup>1</sup>, J. Thomas<sup>1</sup>, J. Zhang<sup>1</sup>; <sup>1</sup>Veterinary Diagnostic and Production Animal Medicine, Iowa State University, Ames, IA, USA, <sup>2</sup>Veterinary and Biomedical Sciences, South Dakota State University, Brookings, SD, USA.

Porcine reproductive and respiratory syndrome virus (PRRSV) is the most economically important disease affecting the swine industry. Modified live PRRSV vaccines often demonstrate poor cross-protection against genetically diverse PRRSV currently circulating in swine. The objective of this study was to evaluate the magnitude of cross-protection in nursery pigs vaccinated with Foster<sup>TM</sup> PRRSV against challenge with a contemporary, heterologous lineage 9 PRRSV isolate. Sixty-three, PRRSV-naïve, mixed breed nursery pigs were randomly allocated into non-

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vaccinated/non-challenged (NV/NC), non-vaccinated/challenged (NV/C), and vaccinated/challenged (V/C) groups. V/C pigs were administered 2ml of Foster<sup>TM</sup> PRRSV intramuscularly at approximately four weeks of age. At 28 days post vaccination, V/C and NV/C pigs were challenged with 2ml of 1x10<sup>5</sup> TCID<sub>50</sub>/ml of a lineage 9 PRRSV (12-39404) both intranasally and intramuscularly. NV/NC pigs were similarly inoculated with sterile cell culture media, and remained PRRSV negative throughout the study. NV/C and V/C pigs demonstrated similar levels of viremia in serum at 3 days post inoculation (dpi); however, virus levels detected in the V/C pigs at 7 and 12 dpi were significantly lower ( $P \leq 0.05$ ) compared to the NV/C group. Gross and microscopic lung lesions were significantly reduced ( $P \leq 0.05$ ) in the V/C pigs compared to the NV/C group. PRRSV 12-39404 was also detected at significantly lower ( $P \leq 0.05$ ) quantities in the V/C bronchoalveolar lavage fluid and lung tissue; this contrasted with significantly higher levels of 12-39404 in tonsil compared to the NV/C pigs. Post-challenge average daily gain (ADG) was significantly higher in V/C pigs compared to NV/C pigs, but significantly lower compared to the NV/NC group. This study demonstrates that Foster<sup>TM</sup> PRRSV provided partial cross-protection against a heterologous challenge by reducing the severity of pneumonia, microscopic lesions, and the level of viremia compared to naïve, challenged pigs. In addition, vaccination conferred the benefit of improved performance post-challenge with a contemporary lineage 9 PRRSV field isolate.

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Mucosal correlates of cross-protection for live-attenuated influenza virus vaccines in pigs.

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Controlling influenza A virus (IAV) in swine has become increasingly difficult with the emergence of novel reassorted strains and introduction of human seasonal IAV into pigs. In North America there are six antigenically distinct H1 subtypes currently circulating in pigs. Live-attenuated influenza virus (LAIV) vaccines provide broader cross-protection than whole-inactivated virus (WIV) vaccines making LAIV a candidate for next-generation swine IAV vaccines. However, a defined immune correlate or standardized assay has not been identified to predict cross-protection following LAIV vaccination. Hemagglutination-inhibiting (HI) immunoglobulin (Ig) in serum has long been the gold standard correlate of protection following WIV vaccination; however, LAIV does not elicit a robust serum HI Ig titer. Oral fluids (OF) have become a rapidly developing diagnostic specimen for a variety of animal pathogens. In order to evaluate mucosal immunogenicity and identify a potential correlate of protection, groups of pigs were vaccinated with different LAIV vaccines encoding pandemic surface genes and subsequently challenged with a heterologous beta-cluster or gamma-cluster IAV. Following vaccination nasal wash (NW) and OF were collected to evaluate Ig levels against a panel of H1 viruses. Both NW and OF had detectable levels of IAV-specific Ig when measured by whole-virus ELISA. Higher IAV-specific IgA endpoint titers were associated with reduced virus shedding following challenge with heterologous IAV. These data suggest OF can serve as a sample for evaluating LAIV immunogenicity and predicting cross-protection.

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PLGA-Nanoparticle entrapped swine influenza virus peptides vaccine induces epitope specific cell-mediated immune response in pigs

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**Purpose:** Pigs are mixing vessels for the emergence of new influenza viruses (IV). The IV conserved peptides have the potential to elicit cross-protective response, but without the potent vaccine delivery system they are poorly immunogenic. Biodegradable poly(lactic-co-glycolic acid) (PLGA) nanoparticle (PLGA-NP) is a potent vaccine delivery system capable of presenting the antigens to the immune system, and also possess the adjuvant property. In this study PLGA-NP entrapped swine influenza virus (SwIV) peptides were tested in a pig vaccine challenge model. **Methods:** Norovirus P particle M2e chimera and conserved four SwIV peptides were entrapped in PLGA-NP by double-emulsion method. Influenza antibody free 4-5 weeks old conventional pigs were vaccinated twice at two weeks interval intranasally, and two weeks post-booster challenged with a SwIV (Sw/OH/24366/07) through intratracheal and intranasal routes; and at 7 days post-challenge euthanized and determined the immune correlates and viral load.

**Results:** The PLGA-NP entrapped peptide vaccine received pigs had no fever in spite of comparable gross lung lesions compared to control virus-challenged animals. Interestingly, though the viral RNA copy numbers in BAL fluid was not significantly reduced in PLGA-NP vaccine group

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compared to control animals, the replicating infective virus was absent in NP vaccine received pigs. Immunologically, the difference in specific antibody, virus neutralizing and hemagglutination inhibition titers though higher in PLGA-NP vaccinated compared to control animals, the data was not statistically significant. But strikingly, the PLGA-NP vaccine received pigs had significantly increased frequencies of IFN- $\gamma$  secreting CD3+CD4+CD8-, CD3+CD4-CD8+ and CD3+CD4+CD8+ cells in the lung mononuclear cells analyzed by flow cytometry. This data was consistent with the secretion of increased amounts of IFN- $\gamma$  in the supernatant of in vitro stimulated lung mononuclear cells.

Conclusions: Our data suggested that the PLGA-NP entrapped candidate SwIV peptides vaccine induced the viral epitope specific cell-mediated immune response in intranasally vaccinated pigs.

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Recovery and Stability of DNA and Protein Antigens Formulated with VaxLiant Adjuvants Utilizing in vitro and in vivo Testing

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A new class of adjuvants capable of being customized to the antigen is being developed by VaxLiant. Stability and recovery studies are being conducted to assess DNA and protein antigens formulated with these adjuvants when stored at refrigerated or ambient temperatures by in vitro antigen recovery and in vivo immune response. Recovery of antigen from adjuvanted vaccines is of primary importance when performing vaccine potency release assays.

Vaccines formulated with plasmid DNA and ENABL 1 or tris-HCL buffer were stored refrigerated and tested at selected time points for 12 months. The plasmid was extracted, quantitated by A260, and subjected to agarose gel electrophoresis. The DNA concentration remained stable and the plasmid was intact after 12 months of storage.

Vaccines formulated with streptolysin O (SLO) and ENABL 1, ENABL 6 or phosphate buffered saline (PBS) were assessed for recovery after refrigerated storage. The protein was recovered and quantitated by ELISA; no extraction method was required. However, for other protein-adjuvant combinations, an extraction process may be required.

Vaccines formulated with bovine serum albumin (BSA) and ENABL 1, ENABL 6 or PBS were assessed in vivo for stability. Female CF-1 mice were inoculated with the vaccines on the day of vaccine preparation and again following 6 months storage at refrigerated or ambient temperatures with a new group of mice. The level of anti-BSA response was measured in the serum collected using ELISA. As expected the immune response to BSA was measurably greater when the BSA was formulated with adjuvant. In addition, titers did not decrease using vaccines stored for six months at either temperature.

These results indicate antigens formulated with ENABL 1 and 6 may be recovered in vaccine potency release assays and to date, the vaccines tested in this study have remained stable at refrigerated and ambient temperatures.

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Bovine ocular and systemic immune responses to an intranasal recombinant *Moraxella bovis* cytotoxin subunit vaccine adjuvanted with polyacrylic acid

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The etiologic agent of infectious bovine keratoconjunctivitis (IBK or 'pinkeye') is *Moraxella bovis*, an organism whose pathogenicity requires the expression of pilin for attachment to the ocular surface and cytotoxin that damages the corneal epithelium. Commercial IBK vaccines currently available in the U.S.A. are labeled for parenteral administration, however, such vaccines are variably effective and there has been increasing interest in the development of mucosally-delivered IBK vaccines. We recently reported significant differences in tear antigen specific IgA between calves vaccinated intranasally with different concentrations of a recombinant *M. bovis* cytotoxin subunit adjuvanted with polyacrylic acid. To further evaluate if systemic and ocular immune responses to an intranasal recombinant *M. bovis* cytotoxin subunit vaccine could be improved by altering the physical state of the vaccinal antigen, 3 groups of steers were vaccinated intranasally with polyacrylic acid plus either saline, an insoluble recombinant *M. bovis* cytotoxin subunit, or a mixture of soluble plus insoluble recombinant *M. bovis* cytotoxin subunit. Tear and serum samples were collected once weekly for 8 weeks and steers were boosted on day 21. Serum and tear cytotoxin (hemolysin) neutralizing antibody responses, tear and serum antigen specific IgG, and tear antigen specific IgA responses were quantitated. Fold changes in these variables from day 0 to days 14, 28, 42, and 55 were calculated. Nonparametric analysis of variance was performed with  $P < 0.05$  as the level of significance. Significantly different fold changes in all measured variables between the 3 vaccine groups were found. Results suggest that an intranasally administered recombinant *M. bovis* cytotoxin subunit antigen adjuvanted with polyacrylic acid can stimulate local and systemic antibody responses against *M. bovis* cytotoxin. Further studies are needed to determine if this vaccine will be effective against naturally occurring IBK.

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Effect of VaxLiant adjuvants on efficacy of mucosal administration of plant-derived Newcastle Disease vaccines in chickens

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Three studies were conducted by Benchmark Biolabs (Lincoln, Nebraska) to evaluate the ability of Newcastle Disease virus (NDV) vaccines to elicit an immune response and protect against challenge, when administered via the mucosal route using various adjuvants from VaxLiant. In the first proof-of-concept study, eleven day old chicks were vaccinated with plant-derived hemagglutinin-neuraminidase (pHN) by controlled dosing via intranasal/intraocular (IN/IO) administration.

Ten of 24 birds demonstrated seroconversion by ELISA after 3 doses, with adjuvants ENABL P1 and experimental BioMize P1 being the most effective (3 of 5 seroconverted from each group), compared to 4 out of 5 birds vaccinated by subcutaneous (SQ) administration. In the follow-up study, ten day old broiler chicks, with various levels of maternal antibodies detectable until day 28 of the study, were vaccinated with pHN by IN/IO administration. A total of 30% and 45% of birds had hemagglutinin inhibition (HAI) titers in groups vaccinated with adjuvants BioMize P1 or ENABL P3, respectively, after 3 doses (day 35). Following challenge with virulent NDV at day 35 post vaccination, 30% and 20% of the birds in those groups were not protected, compared with 67% of negative controls. In the final study, seven day old NDV-seronegative chicks were vaccinated with pHN by IN/IO administration. Birds vaccinated by SQ administration demonstrated 83% seroconversion after a single dose,

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while IN/IO groups vaccinated with adjuvant ENABL P3 had up to 50% seroconversion after 2 doses. Following challenge at day 29, 17% of the birds were not protected, compared to 100% of the negative control birds and 0% of the SQ birds.

The results from this series of studies demonstrates that 1) plant-derived antigen elicited an immune response and protected against virus challenge, 2) vaccines administered via the oculonasal route were effective, although not to the same level as SQ administration using the present adjuvant formulation, and 3) proprietary VaxLiant adjuvants BioMize P1, ENABL P1, and ENABL P3 provided different levels of protection depending on the study, indicating opportunity to formulate to the target antigen and method of immunization.

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Challenges with Poultry Immunology and Disease Research: Immune reagent development and maintaining genetic breeds of poultry  
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A major obstacle to advances in veterinary and disease control is the lack of sufficient immunological reagents specific for ruminants, swine, poultry, equine and aquaculture species. USDA-funded consortium for veterinary immune reagent development was established in 2004 and this presentation will summarize recent progress in poultry immune reagents development. This veterinary immune reagent network (VIRN) addresses the needs of the following groups: ruminants (concentrating on cattle), swine, poultry (primarily chickens but some evaluation of turkeys), horses and aquaculture species (concentrating on channel catfish and salmonoid trout, two of the principal economically important species). These reagents will be used : i) to determine the cause of immunopathology associated with particular infectious and inflammatory diseases and devise intervention strategies, and ii) to develop protective vaccines against infectious diseases to improve animal health. The reagents will benefit a large group of US and international researchers including veterinary immunologists, pathologists and microbiologists.

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Propagation of feline respiratory epithelial cells at the air-liquid interface - an in vitro model to study felid herpesvirus 1 in cats  
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Felid Herpesvirus type 1 (FHV1) causes ~ 50% of diagnosed viral upper respiratory tract disease in cats. Despite vaccinations, the virus spreads rapidly, frequently leading to euthanasia. This incomplete protection by vaccination is largely attributed to the immunosuppressive properties of the virus. Because the first viral/host interaction occurs at the respiratory tract epithelium, respiratory epithelial cell culture models have been developed in humans and animals to study the virus/cell interactions at this site. These cultures have shown to resemble the natural airway. However, to date no such air-liquid interface (ALI) cultures have been developed in cats. The purpose of this study was to establish and characterize ALI-feline respiratory epithelial cell cultures (FREC), and investigate whether ALI-FRECs support FHV1 infection. Respiratory tracts from 4 cats were collected and epithelial cells were isolated from the upper, middle, lower trachea and bronchus and cultured at the ALI. Sections of ALI-FRECs and the natural airway were compared histologically and immunologically. Gene expression profiles of 36 genes (toll-like receptors, cytokines, chemokines and antiviral genes) were compared between ALI-FRECs, freshly isolated FRECs and whole tissues. Furthermore, ALI-FRECs were infected with FHV1 to show that these cultures support infection and viral replication. Morphological ALI-FRECs resembled the natural airway with multilayered pseudostratified ciliated epithelial cells secreting mucus. In addition, gene expression of toll-like receptors, cytokines, chemokines and antivirals was detected in ALI-FRECs and the expression profiles were similar to the natural airway and freshly isolated FRECs. Finally, we found that ALI-FRECs supported infection and replication with FHV1. Our results suggest that ALI-FRECs mimic the natural airway by retaining the majority of the morphological and immunological properties and supporting infection with FHV1. In the future ALI-FRECs will facilitate a comprehensive investigation of cell-viral and cell-cell interactions and allow for development of novel mucosal vaccines and adjuvants.

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The role of interferon in clearing primary Bovine Herpesvirus-1 (BHV-1) infections  
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Bovine respiratory disease is an important clinical problem and primary BHV-1 respiratory infections are a frequent component of this disease complex. Primary BHV-1 infections induce high levels of interferon (IFN)- $\alpha$  and - $\gamma$  in nasal secretions but IFN-treatment of calves did not reduce viral shedding. We hypothesize that with high IFN levels, BHV-1 escapes this potent antiviral defense by inhibiting IFN-induced antiviral effectors at either a transcriptional, translational or post-translational level. Pre-treatment of cultured bovine epithelial cells with bovine IFN- $\alpha$ I and - $\gamma$  revealed that BHV-1 is over 1000-fold more resistant to IFN antiviral activity than Vesicular Stomatitis Virus. This IFN resistance was conserved among plaque-purified BHV-1 isolates. Gene expression analysis, using qRT-PCR, confirmed that bovine IFN- $\alpha$ I and - $\gamma$  rapidly induced sustained expression of Mx1, OAS, RNase L and BST-2 antiviral effector genes. BHV-1 infection of IFN treated epithelial cells did not inhibit transcription of the interferon-induced antiviral effector genes. This observation suggested BHV-1 may inhibit IFN-induced antiviral effector proteins at translational or post-translational level. In contrast, qRT-PCR analysis of IFN-induced effector gene expression in nasal turbinates and trachea revealed a specific down-regulation of RNase L following primary BHV-1 infection. RNaseL is an important inhibitor of DNA virus replication. Therefore, in vivo, BHV-1 may effectively evade IFN-induced antiviral effector mechanisms at a transcriptional level. This raises a critical question regarding the immune mechanism mediating BHV-1 clearance between 7 to 10 days post-infection. Immunohistochemical staining of tissue sections from the upper respiratory tract (URT) revealed increased recruitment of NK cells and CD8+ T cells between days 5 to 7 post-infection. Dual staining is in progress to determine if NK cells or CD8 T cells are the source of IFN- $\gamma$  detected in nasal secretions. Identifying BHV-1 immune evasion strategies and immune responses mediating BHV-1 clearance will provide better surrogate markers for evaluating the safety and efficacy of new vaccine candidates.

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Late-gestational nutrient restriction affects immune responsiveness of offspring in beef cattle.

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In many species the nutritional status of the dam during pregnancy can affect the long-term health of her offspring. Beef cows are often undernourished during gestation because of poor forage nutrient availability and the increasing nutrient demands of pregnancy. Early health and growth of calves is of utmost importance to cow-calf producers, many of whom market calves shortly after weaning. Therefore, better understanding of immunologic consequences of maternal nutrient restriction will allow for improved management of prepartum cows and postnatal calves. A late gestational forage system model was used to evaluate effects of nutrition during pregnancy on offspring immune function. Mature beef cows were either fed poor-quality tall fescue hay (6.2% CP, low digestibility) or allowed to strip-graze stockpiled tall fescue pasture (12.3% CP, average digestibility) during the last 95 days of gestation. Calves born to hay-fed dams had reduced birth weights, suggesting impaired fetal growth. Immune response of 8 calves from each treatment was assessed by ex-vivo whole blood stimulation with low and high concentrations of 3 Toll-like receptor agonists (lipopolysaccharide, lipoteichoic acid and peptidoglycan). Stimulations were incubated for 4 hours at 35°C. Following stimulation, expression of the following genes was assessed using RT-PCR: interleukin-1 (IL-1), interleukin-6 (IL-6), interleukin-8 (IL-8), inducible nitric oxide synthase (iNOS) and tumor necrosis factor alpha (TNF $\alpha$ ). Results suggest that calves born to hay-fed dams have a more robust pro-inflammatory response to stimulation at 48 hours of age; this was most evident with respect to IL-1 expression. Stimulations performed at 3 months of age showed no differences due to maternal treatment. Despite this, a growth disparity remained where calves born to hay-fed cows tended to have decreased rates of gain. A more pro-inflammatory phenotype could be either beneficial or detrimental to the overall health of these calves; however, it may indicate that different strategies are needed to prevent and treat disease in calves born to nutrient restricted dams.

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Immune suppression in BLV-infected dairy cattle

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Bovine leukemia virus (BLV) is a retrovirus that infects cattle and is particularly prevalent in US dairy herds. Over 83% of dairy herds are infected and the within-herd prevalence often exceeds 30%. BLV infection negatively impacts the dairy industry due to decreased milk production and increased culling rates. However, BLV may have a more serious impact on dairy production due to immune suppression and an increased risk for developing other infectious diseases. To further examine the effects of BLV infection on immune reactivity, PBMCs were isolated from naturally infected BLV+ Holstein cattle and healthy controls and cultured for 2 days in the presence or absence of pokeweed mitogen (PWM). Indirect immunofluorescence was used to characterize both the relative percentage of T cell subsets, B cells and monocytes, and the degree of activation in the presence of mitogenic stimulation. BLV-infected cattle demonstrated atypical responses to stimulation, providing further *in vitro* evidence of immune dysregulation in BLV-infected dairy cattle.

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Effects of conditioned media from *Histophilus somni* infected bovine brain endothelial cells on fibrin deposition and Factor Xa activity of bovine neutrophils

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*Histophilus somni* is a gram negative coccobacillus that causes respiratory, reproductive and central nervous system disease in cattle. The hallmark of *H. somni* infection is diffuse vasculitis and intravascular thrombosis that can lead to an acute central nervous system disease known as thrombotic meningoencephalitis (TME). Because neutrophils are major players in the pathophysiology of septic meningitis, we sought to determine their role in *H. somni* induced fibrin clot formation *in vitro*. Bovine Brain Endothelial cells (TBBE cells) were inoculated with *H. somni* (25:1 multiplicity of infection) and incubated for 6 hr at 37°C with 5% CO<sub>2</sub>. Conditioned media (CM) were collected, centrifuged at 800 g for 15 min at 4°C, and filtered through a 0.22  $\mu$ m filter. Freshly isolated peripheral blood polymorphonuclear neutrophils (PMNs) were incubated for 6 hrs with various concentrations of CM from *H. somni* infected TBBE cells, or uninfected cells as a control. PMNs were washed twice with Ca<sup>2+</sup> and Mg<sup>2+</sup> free PBS, and fibrin clot formation and tissue factor activity assessed by a recalcified plasma clotting assay. We found greater tissue factor activity on PMNs and their lysates after incubation with CM from *H. somni*-infected than uninfected control TBBE cells. In addition, PMNs exposed to CM from *H. somni*-infected TBBE cells exhibited increased fibrin clot formation, and Tissue Factor activity and expression as compare to uninfected control. Our results suggest that bovine PMNs might amplify thrombus formation in bovine brain microvasculature during *H. somni* infection and by so doing contribute to the process that results in TME.

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Expression of inflammation-associated genes in circulating leukocytes and activity of indoleamine-2,3-dioxygenase in dairy cattle with acute puerperal metritis and bacteremia.

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The objective of this study was to investigate whether expression of genes associated with inflammation and activity of Indoleamine-2,3-Dioxygenase (IDO) correlated with disease status and prevalence of bacteremia in post-partum dairy cattle. Blood was collected from cattle with APM and control cattle matched by parity and days in milk. Leukocytes were isolated, diluted to a standard concentration, and frozen until RNA extraction. Expression of 6 genes associated with inflammation (TNF- $\alpha$ , IL-1 $\beta$ , IL-4, IL-6, IL-10, and IDO) was quantified by use of a real-time quantitative reverse transcription PCR assay. Serum was collected after centrifugation of whole blood and frozen until analysis by HPLC. Results were grouped by disease status and presence of bacteremia for comparison.

The relative expression of IL-1 $\beta$  in cattle with APM was significantly lower than that in controls. No significant difference was found in the relative expression of other genes tested. Serum levels of tryptophan and kynurenine were not significantly different between cattle with APM and controls. Activity of IDO was not significantly different between cattle with APM and controls. Serum levels of tryptophan, kynurenine, and IDO activity were not significantly different between bacteremic cattle and non-bacteremic cattle. The expression of IL-1 $\beta$  was lower in cattle with APM. Otherwise, cytokine and IDO gene expression were not significantly different between cattle with APM and healthy controls, as well as bacteremic and non-bacteremic cattle. The activity of IDO in serum of cattle with APM and healthy controls, as well as bacteremic and non-bacteremic cattle, was similar. The lower levels of IL-1 $\beta$  expression in PBMCs of cattle with APM suggest impaired inflammatory responses and may contribute to the development of the disease in this population of animals.

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Oxylipid profiles in biological samples of dairy cows with coliform mastitis.

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Coliform mastitis is characterized by acute robust inflammation that causes severe mammary gland pathology, decreased milk production and fatal sepsis in dairy cattle contributing significantly to economic losses to the dairy industry. Oxylipids are potent lipid metabolites, synthesized from polyunsaturated fatty acids (PUFAs) released from cell membrane phospholipids, which can regulate the initiation, progression and resolution of inflammation. The imbalance between pro- and anti-inflammatory oxylipids determines the outcome of several inflammatory based diseases. Although a few pro-inflammatory oxylipids were reported during bovine mastitis, comprehensive lipidomic analyses that include the many metabolites that comprise the extensive oxylipids network have not been performed. Therefore the objective of this study was to define the oxylipid profiles and changes in the balance between pro- and anti-inflammatory species during coliform mastitis. Milk and plasma samples from dairy cows with systemic coliform mastitis and matched mid-lactation healthy controls were analyzed for fatty acid and oxylipid profiles using liquid chromatography and tandem mass spectrometry. All PUFAs analyzed were elevated with linoleic acid being the most prevalent in milk during coliform mastitis. Similarly, PUFAs were elevated in plasma, but at lower concentrations than milk during coliform mastitis. More pro- and anti-inflammatory oxylipid species were significantly elevated in milk than plasma of coliform mastitis cows. In contrast to milk of coliform infected cows, several oxylipids were significantly elevated in plasma of mid-lactation control cows. In conclusion, the several oxylipid species detected in milk and plasma suggested involvement of multiple enzymatic and nonenzymatic biosynthetic pathways involved in lipid metabolism during coliform mastitis. Greater disruption in oxylipid balance occurred locally in the mammary gland and novel targets should be developed targeting the metabolism of these detected oxylipids especially anti-inflammatory species. The significance of oxylipid profiles in plasma of control healthy cows should be investigated further.

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Bovine neutrophils produce neutrophil extracellular traps (NETs) in response to *Salmonella* serotype Typhimurium

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**Purpose:** In the U.S it has been estimated there may be as many as 1 million salmonellosis cases each year. Most human salmonellosis cases are caused by contaminated food or water. Cattle are susceptible to salmonellosis and can shed the organism in their feces which in turn can contaminate water and various food products. Recently there has been increasing concern about *Salmonella* colonization of lymph nodes in healthy cattle, from which it can enter ground beef. Host defense against *Salmonella* and other bacterial pathogens relies on neutrophils and macrophages, which are important players in innate immunity. One way by which these cells combat bacterial pathogens is through release of DNA, studded with antimicrobial proteins, to form extracellular traps (ETs) that ensnare and kill pathogens. In this study we examined whether *Salmonella* Typhimurium triggers extracellular trap (NET) formation by bovine neutrophils and the fate of *Salmonella* cells caught within these traps.

**Methods:** NET formation was triggered by incubating bovine neutrophils with logarithmically grown *Salmonella* Typhimurium. PicoGreen was used to quantify extracellular DNA. DNase I was used to cleave DNA as a control. Fluorescently labeled and non-labeled *Salmonella* cells were incubated with bovine neutrophils to determine if *Salmonella* were physically trapped and killed by NETs.

**Results:** We find that bovine neutrophils produce NETs in response to *Salmonella* Typhimurium. Apoptosis and necrosis were not significant contributors to NET formation. However, NETs ensnared few cells of *Salmonella* and did not exhibit the ability to kill *Salmonella* Typhimurium despite NET formation. These findings were confirmed by scanning electron microscopy.

**Conclusions:** These data indicate that bovine neutrophils release NETs in response to incubation with *Salmonella* Typhimurium, but are unable to trap or kill *Salmonella* cells. Future research will investigate the mechanism that *Salmonella* uses to evade NETs, in an attempt to better understand how *Salmonella* interacts with the innate immune system.

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The Microbiota-Gut-Brain Axis: Increasing recognition of its role in health and disease

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Recent studies from a number of laboratories have demonstrated that the ability of the microbiota to play an active role in host health, ranging from behavior to susceptibility to infectious disease, is far larger than previously suspected. One of the possible mechanisms by which such interactions between the microbiota and the host can occur is through the shared recognition and production of neurochemicals. The ability of microorganisms that constitute the microbiota to recognize and produce neurochemicals that can influence the host, known as microbial endocrinology, suggests that there is an evolutionary basis by which microbiota and host communicate. It is generally believed that such neurochemicals belong exclusively to the animal kingdom and that any role such neurochemicals play in behavioral or infective processes is largely confined to host physiology and -related parameters. This, however, is wholly incorrect as numerous bacterial species that inhabit the gut have been known for decades to possess the ability to synthesize neurochemicals that are exactly the same in structure (and by the same biosynthetic pathways) as produced by the host. This implies that such shared neurochemicals also allows for bi-directional communication between host and microbiota. The implications of such bi-directional communication are increasingly being recognized to play a role in health and disease.

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Recovery of the gut microbiota is disturbance-dependent

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The interactions of the intestinal microbiota of mammals with the immune system are numerous and complex. With each large animal gut comprising at least 500 different bacterial species and over a trillion cells, the characterization of these interactions is a challenge. To improve our understanding of a healthy gut microbiota, we study the effects of applied disturbances, such as *Salmonella* exposure and antibiotic administration, on swine intestinal bacterial communities. We use highly parallel sequencing of the 16S rRNA gene to determine bacterial

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community membership across individuals, treatments, and time. Our results show that each disturbance has a unique impact on the gut microbiota, and that the recovery of the microbiota to its original state is not guaranteed. Inoculating pigs with the foodborne pathogen *Salmonella enterica* serovar Typhimurium, for example, results in individuals shedding various amounts of *Salmonella*. The microbiota of the low-shedder versus high-shedder pigs differed before and 2-days post-inoculation. However, by 21-days post-inoculation, the microbiota of pigs that had received *Salmonella* were not different from each other, but were different from non-inoculated pigs. These results suggest that the microbiota was altered by *Salmonella* regardless of the degree of carriage, and that the recovery of the microbiota was incomplete by three weeks post-inoculation. In contrast, analysis of the microbiota of pigs that were continuously fed the in-feed antibiotic carbadox (50 g/ton) for three weeks revealed different community dynamics. Carbadox disturbed the bacterial community within two to four days of administration, but the community structure recovered by two weeks post initiation despite the enduring presence of carbadox. The results improve our knowledge of the gut microbiota and how it is modulated, and advance the development of non-antibiotic feed additives to modulate the microbiota toward improved animal health and food safety.

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Developing tools to investigate the swine-associated butyrate-producing microbiota and its relationship to *Salmonella* shedding phenotype  
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*Salmonella* is one of the most prevalent foodborne pathogens in the United States, with over half of U.S. swine farms testing positive for *Salmonella*. Consequently there is great interest in developing techniques to control its persistence and spread, including modulation of the swine gut microbiota. Our previous research has shown that the composition of the swine intestinal bacterial community before *Salmonella* inoculation significantly affects the future shedding status of pigs. We hypothesize that differences in the butyrate-producing bacterial community could lead to different *Salmonella* shedding phenotypes. Primers designed for the human butyrate-producing community are available but fail at identifying many cultured swine-associated butyrate producers. To investigate our hypothesis it was necessary to develop primer sets better suited for the swine-associated community. We isolated 15 strains of butyrate-producing bacteria from the swine intestine. We sequenced the genomes of unique strains and identified the functional genes responsible for butyrate production. All 8 unique strains contained Butyryl-CoA: Acetate CoA-transferases (*but*). Degenerate primers were designed to amplify all identified swine *but* genes. Preliminary tests of the swine-specific *but* primers with swine gut bacterial DNA showed improved amplification of the *but* gene over *but* primers designed for butyrate producers within the human microbiome. These primers will enable investigations of the butyrate-producing community from pigs shedding different levels of *Salmonella* in feces. This project will define a functional aspect of bacterial communities that are associated with lower *Salmonella* transmission, and will be an early step toward modulating the microbiota to improve food safety.

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Tailoring probiotics as immunomodulators to enhance neonatal mucosal immunity to rotavirus (RV) vaccines or alleviate RV diarrhea: Evaluation in a neonatal gnotobiotic piglet model.

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RV is a major enteric pathogen of infants and neonatal animals. Attenuated oral RV vaccines for animals often fail in the field, or vaccines lack efficacy in infants in impoverished regions where most needed. Alternative affordable strategies are needed to reduce RV diarrhea and to enhance vaccine efficacy. Probiotics promote immune maturation and mediate beneficial health effects, but mechanisms are largely undefined. We hypothesized that certain probiotics are immunostimulatory and enhance immune responses to vaccines, whereas others are immunoregulatory and moderate RV diarrhea to restore intestinal homeostasis. We elucidated the impact of colonization with single or dual key Gram+ (lactobacillus or bifidobacterial strains) or Gram- (commensal *E. coli*) probiotic bacteria or a commensal cocktail on: 1) maturation of neonatal immunity; 2) moderation of RV diarrhea; and 3) enhancement of RV vaccine efficacy in the neonatal gnotobiotic piglet model. All probiotics tested promoted immunomaturational. Probiotic-colonized, RV vaccinated piglets had reduced RV shedding or diarrhea post-challenge and enhanced gut IgA antibody responses to RV. Overall, dual colonization with selected Gram+ probiotics was more effective at promoting these effects than single Gram+ probiotics. The Gram- *E. coli* commensal alone was more effective than the Gram+ probiotics alone or combined. Each probiotic strain had both common and divergent transcriptomic profiles, indicative of regulation of diverse pathways. Thus probiotics, alone or combined, exerted divergent immune modulating effects when interacting with attenuated RV oral vaccine (enhanced T and B cell responses) versus enteropathogenic RV (enhanced Tregs and anti-inflammatory cytokines). In future studies, we will transplant human microflora into gnotobiotic piglets to investigate interactions among probiotics, the human gut microflora, antibiotics, enteric viral pathogens and oral vaccines. Our goal is to identify potential biomarkers that will enhance oral vaccines, or therapeutic interventions for viral diarrheas.

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Directed Evolution Of An Adeno-Associated Virus Library *In Vivo* Pig Airway.

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Patients with cystic fibrosis (CF) develop persistent respiratory symptoms and progressive airway infection and inflammation. Gene transfer offers the potential to express CFTR in the lungs of patients and prevent disease. Adeno-associated viruses (AAVs) are members of the Parvovirus family. Aerosol of an AAV2 vector expressing CFTR airways of patients with CF have led to no significant safety concerns. However, inefficient transduction by AAV2 from the apical surface is major problem. We performed directed evolution of an AAV library in pig airways *in vivo*. This strategy identified a single AAV capsid variant (AAV2H22). AAV2H22 differs from AAV2 by only five amino acid residues; two are exposed on the capsid surface (Q598L and V708I), the remaining three are not (E67A, S207G, and I648V). We found AAV2H22 efficiently and selectively transduce pig airway epithelia (PAE). Moreover, evaluation of the individual mutations in AAV2 by site-directed mutagenesis suggests that AAV2H22 has solved several rate limiting steps for transduction. Mutagenesis of the surface exposed residues Q598L and V708I in AAV2 is sufficient to enhance gene transfer to PAE, yet neither mutation is required. Moreover, AAV2 with the V708I mutation alone, or reverting Q598L to a Q in AAV2H22, outperformed AAV2H22. This suggests that some mutations were selected for functions

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other than their ability to infect PAE. Additionally, the V708I mutation may have added advantages *in vivo* as it may minimize neutralization by antibodies. We also found that E67A (a residue is close to the PLA2 catalytic domain) enhances AAV2-mediated gene transfer but is not required. Finally, I648V alone enhances gene transfer of AAV2 and the reversion mutant impairs gene transfer by AAV2H22. This mutation is in an interloop region of unknown function. In summary directed evolution of an AAV library in pig resulted in an efficient gene transfer vector that can be used to investigate if gene therapy in newborn CF pigs can prevent lung infections.

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Evaluating the metagenome of nasal samples from cattle with bovine respiratory disease complex (BRDC)

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Bovine respiratory disease complex (BRDC) is an involved multi-factor disease, which is the most expensive animal disease afflicting herds in U.S. beef cattle industry, costing the industry over \$1 billion annually. Animals may be predisposed to suffer from BRDC by a variety of viral and bacterial infections and it is increasingly evident that disease incidence is intimately associated with an animal's commensal microbiota (metagenome). Therefore, evaluation of the animal's resident microbiota in the upper nasal cavity may help us to understand the impact of the metagenome on incidence of BRDC in cattle. To determine the microbiota from the upper respiratory tract of cattle diagnosed with BRDC, nasal swabs from the upper nasal cavity were collected when the animal was diagnosed with BRDC. These were taken approximately one, three, and four weeks after weaning at the feedlot. Samples from healthy cohorts were also collected at the designated time points as controls. Nasal swabs from ten animals were collected and pooled for DNA extraction at each time point within each phenotype (sick or control), for a total of 6 pools. To evaluate and compare the metagenome of each pooled sample, the variable region (approximately 1,500 bp) along the 16S ribosomal RNA gene was amplified by PCR to include v1-v8. These amplified products were then sequenced using next-generation sequencing (Pacific Biosciences RSII instrument, Pacific Biosystems, Menlo Park, CA) and sequence reads were analyzed by WebMGA to identify subfamilies for the bacterial populations present. Overall, Mannheimia haemolytica was the predominant bacterial subfamily present in all pools evaluated (34-87%), with the BRDC (sick) pools having a greater percentage of Mannheimia haemolytica compared to the control pools at week 1 and week 4 (week 1: 87% versus 44%; week 4: 58% versus 35%). These results confirm the likely role of Mannheimia haemolytica in BRDC.

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Evaluation of changes in VapA-specific IgG and IgG subclasses over time to identify foals with Rhodococcus equi pneumonia

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Rhodococcus equi (R. equi) is a common cause of pneumonia in foals; however early diagnosis remains a challenge. While serology may be of use, the humoral response to this disease is undefined. The objectives of this study were 1) To evaluate changes in VapA-specific IgG, IgGa, IgGb and IgG(T) over time after experimental infection, natural exposure and natural infection of foals with R. equi and 2) To evaluate the performance of a VapA-specific ELISA to detect early diagnosis of pneumonia caused by R. equi. Immunoglobulin changes over time were described using sera from foals experimentally challenged with R. equi at 1 (n=18), 2 (n=4) and 3 (n=6) weeks of age and in foals naturally exposed (n=126) or naturally affected with rhodococcal pneumonia (n=15). The diagnostic performance of the ELISA was evaluated using sera from known positive (n=31), healthy but exposed (n=125) and non-exposed (n=10) horses. To evaluate IgG changes over time, repeated measures ANOVA on ranks was used and significance was set at  $p < 0.05$ . For each VapA-ELISA evaluated, a cut-off value was selected based on ROC curve analysis and used to calculate sensitivity, specificity and positive and negative predictive values.

Following birth, VapA-specific IgGs significantly ( $p < 0.05$ ) decreased over time in all foals as a result of normal decay of passively transferred antibodies. Both VapA-specific IgGa and IgG(T) significantly increased ( $p < 0.05$ ) beginning at 5 weeks of age in foals that developed rhodococcal pneumonia after experimental and natural challenge. VapA-specific IgGb also increased over time in foals that remained clinically normal after exposure to R. equi. Thus, when used to identify infected foals, VapA-specific IgG, IgGa and IgGb had no diagnostic value. In contrast, IgG(T) had good sensitivity, specificity, positive and negative predictive values.

In conclusion, VapA-specific IgG subclasses, with the exception of IgG(T), are poor predictors of disease due to the presence of antibodies caused by environmental exposure. Further investigation of the use of IgG(T) as a diagnostic tool under field conditions is needed.

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The effect of passively-acquired antibodies on Lawsonia intracellularis infection and immunity in the horse

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Multiple hypotheses into the age-based susceptibility of horses to Lawsonia intracellularis exist. The objective of this study was to determine whether the decline in passively-acquired antibodies in horses is responsible for the age predilection of equine proliferative enteropathy (EPE), caused by L. intracellularis. Additional objectives included examination of various risk factors for the development of EPE as well as the determination of naturally-occurring attack rates for clinical and subclinical EPE. A total of 369 mare and foal pairs from 15 central Kentucky Thoroughbred farms were used in this study, which took place from January 2012 through February 2013. Serum samples were collected from mares and foals within 48 hours of parturition, and then monthly from foals to February of their yearling year. L. intracellularis-specific antibodies were measured using an ELISA. No effect of passively acquired antibodies on the occurrence of presumptive clinical or subclinical EPE was noted. In total, 5.3% and 6.3% of seropositive horses developed presumptive clinical or subclinical EPE, respectively. In multiple logistic regression models, colts were at a significantly greater risk than fillies of developing presumptive clinical EPE ( $p = 0.038$ ) or a combination of either presumptive clinical or subclinical EPE ( $p = 0.006$ ) and foals that were weaned in September or beyond were at a lower risk of developing presumptive EPE ( $p = 0.047$ ). This study is the first to show that passively-acquired antibodies to L. intracellularis do not protect against clinical or subclinical EPE. A number of novel findings, including identification of the disease rate amongst naturally exposed horses, warrant additional work as they may help to identify potential risk factors for L. intracellularis exposure and/or the reservoir host(s) of the bacterium.



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Most Prevalent Poultry-associated *Salmonella* SeroTypes differ in their susceptibility to widely used carcass sanitizer, chlorine  
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Poultry-associated non-typhoidal *Salmonella* serotypes are the important cause of food-borne illnesses in humans. Despite aggressive use of different FDA approved sanitizers in poultry processing, significant proportion of marketed poultry meat in the US remains contaminated with few Most Prevalent Poultry-associated *Salmonella* serotypes (MPPSTs). These include Typhimurium, Enteritidis, Heidelberg, Montevideo, Mbandaka, Seftenberg, Kentucky and Infantis. Our hypothesis is that there is inter- or intra-strain differences in the resistance of different MPPSTs against the widely used carcass sanitizers. The specific objectives of this study were to (i) develop a food-based model that simulates carcass chilling; a major step in commercial carcass processing and (ii) determine the differences in susceptibility of different MPPSTs against chlorine, the most widely used carcass sanitizers. To develop a food-based model, chlorine (Clorox®) was added to the ice cold (4°C) water (pH 4.0, adjusted with SAS®) to a final concentration of ~50 PPM. A filter sterilized chicken meat extract (CME) was collected from frozen chicken carcasses and added to chlorinated water to a final concentration of 1, 2, 3, 4, 5, 10, 15 and 20% (v/v) to resemble the organic environment within the immersion chilling tank. This model was challenged with  $5 \times 10^4$  CFU of different MPPST strains and their survival was tested at 5, 30, 60 and 90 min post-inoculation. Additionally, the pH, concentration of free and total chlorine was tested at each time point. Irrespective of concentration of CME, the amount of free chlorine decreased from 50 ppm to 3-5 ppm in < 5 min. In general, as the CME concentration increased, the pH of the chlorinated water and the survival of different MPPST strains also increased. At lower CME concentrations (3%), serotypes Kentucky and Mbandaka survived until 5 min; serotypes Heidelberg survived until 30 min whereas Montevideo, Typhimurium, Infantis Seftenberg and Enteritidis survived until 90 min post-inoculation. These data suggest that MPPSTs differ in their susceptibility to chlorine and that the level of CME in immersion chilling is an important contributing factor in *Salmonella* survival.

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A randomized trial to assess whether enrofloxacin metaphylaxis for bovine respiratory disease affects fecal shedding of *Salmonella* and *Campylobacter* in feedlot cattle

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The study objective was to determine whether enrofloxacin metaphylaxis affected the prevalence and fluoroquinolone susceptibility profiles of *Salmonella* and *Campylobacter* recovered from feces of feedlot cattle. Feeder cattle at high risk for bovine respiratory disease (n=288) were randomly assigned to either pens (n=12) that received enrofloxacin (Baytril® 100; 7.5 mg/kg) or non-treated pens (n=12). Rectal fecal samples were collected from animals on days 0, 7, 14, 21, and 28 (+/- 2 days). Cattle clinically ill and requiring antibiotic therapy were treated and removed from the study. Fecal samples were cultured for *Salmonella* and *Campylobacter* using selective enrichment methods; presumptive isolates were confirmed using latex agglutination and PCR. Bacterial susceptibility to ciprofloxacin and naladixic acid was evaluated using the Clinical and Laboratory Standards Institute broth micro-dilution methods and human breakpoints. Data were analyzed using linear mixed models. Of the 1,388 samples collected across all study days, 10.1% (140) were positive for *Salmonella* and 12.4% (172) were positive for *Campylobacter*. *Salmonella* prevalence was not significantly different among treatment groups ( $P = 0.95$ ), but was significantly different across study days ( $P < 0.01$ ) with prevalence decreasing over the study period. *Campylobacter* prevalence was not significantly different among treatment groups ( $P = 0.20$ ), nor was it significantly different across study days ( $P = 0.06$ ). At the beginning of the study (Day 0), 98.9% (92/93) of the *Salmonella* isolates recovered across both treatment groups were susceptible to naladixic acid and ciprofloxacin. For all subsequent sampling days, 100% of the isolates (n=47) were susceptible to both drugs. *Campylobacter* susceptibility testing and statistical analysis are currently underway. Preliminary results indicate that metaphylactic administration of enrofloxacin in feedlot cattle did not significantly affect fecal prevalence of *Salmonella* and *Campylobacter*. Additionally, metaphylactic administration of enrofloxacin did not impact naladixic acid or ciprofloxacin susceptibilities of *Salmonella* isolated from feeder cattle feces.

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Characterization of a Putative Hemolysin of *Campylobacter jejuni* for Potential Use in Vaccination of Poultry.

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*Campylobacter* is the most common cause of bacterial gastroenteritis worldwide and the second most common within the U.S., with an annual estimated cost of \$1.2 billion. Up to 80% of these human infections are attributable to the poultry reservoir. Risk assessment indicates that just a 2-log reduction of the *Campylobacter* load on chickens would reduce the incidence of campylobacteriosis associated with chicken meals by a factor of 30. Currently, no interventions are available to the poultry industry which would allow this goal to be achieved, however, vaccination is a promising strategy. Our group has previously utilized a recombinant attenuated *Salmonella* vaccine (RASV) vector to achieve reduction of *C. jejuni* colonization of poultry. In this study, we evaluated the potential of a putative hemolysin of *C. jejuni* for use as a possible vaccine antigen. Purified protein was created by overexpression in *Escherichia coli*. The reaction of sera and intestinal immunoglobulin from chickens experimentally colonized by *C. jejuni* was examined for reactivity against the purified protein. Additionally, mucosal and serum responses following subcutaneous and oral vaccination of chickens using purified protein were assessed.

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Plasmids of enteric bacterial pathogens: past, present, and future challenges

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Plasmids and other mobile genetic elements are key agents of change in bacterial populations. Plasmids have a long history associated with molecular biology and antibiotic resistance; however, the genome sequencing era has finally enabled us to better appreciate plasmid diversity and evolution. Among Enterobacteriaceae, a number of plasmids exist that are implicated in virulence and/or antimicrobial resistance. Some of these plasmids are of narrow host range, whereas others possess apparently broad host range. This presentation will provide an overview of the key genetic differences between plasmid types among Enterobacteriaceae, and work in our laboratory aimed at understanding the regulatory interactions enabling the success of broad-host-range plasmids.

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Pooling of immunomagnetic separation beads does not affect sensitivity of detection of seven serogroups of Shiga toxin-producing *Escherichia coli* in cattle feces

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Shiga toxin-producing *Escherichia coli* of serogroups O26, O45, O103, O111, O121, O145 and O157 (STEC-7) are major foodborne pathogens. Cattle are a major reservoir of STEC and shed the organisms in the feces, which serves as a source of contamination of food products. For culture-based detection of STEC, feces are enriched in a selective broth and subjected to immunomagnetic separation (IMS) with serogroup-specific beads. Bead suspensions are then spread-plated onto selective medium and colonies are tested by PCR for serogroup and virulence gene confirmations. The IMS procedure is time consuming and labor intensive because of the need to subject each fecal sample to seven individual beads. Therefore, our objective was to evaluate whether pooling of IMS beads affects detection sensitivity of STEC-7 compared to individual IMS beads. Fecal samples from cattle were spiked with seven STEC individually or in pooled combinations (pools of 3, 4, 6 or 7) before (n=6) or after (n=6) enrichment in *E. coli* broth for 6 h at 40° C and subjected to individual and pooled IMS beads. Beads were spread-plated onto sorbitol MacConkey with cefixime and tellurite (CT-SMAC; for O157), and Possé medium modified to include novobiocin at 5 mg/l and potassium tellurite at 0.5 mg/l (MP; for six non-O157). Up to six (for individual beads) or ten (for pooled beads) presumptive colonies from MP and CT-SMAC were tested by multiplex PCR to confirm serogroups. Recovery of STEC-7 from spiked feces with pooled beads was comparable to the recovery with individual IMS beads. Cattle feces (n=384) from feedlots were evaluated for detection by individual or two combinations of pooled non-O157 IMS beads (O26+O45+O111 and O103+O121+O145). Non-inferiority tests indicated that pooling IMS beads was not substantially inferior ( $P < 0.05$ ) to individual IMS beads for detecting the six non-O157 serogroups in fecal samples based on non-inferiority margins as low as 5%. The significance of the test was verified by the lower limit of the 90% confidence interval being greater than the non-inferiority limit in all tests for all serogroups. Pooling of IMS beads is advantageous because of reduced time, labor and expense required to detect and isolate STEC-7.

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Transcriptional profiling of *Salmonella* Enteritidis strains identifies genes consistently highly expressed in biologically relevant microenvironments

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Purpose: *Salmonella* Enteritidis (SE), a causative agent of food-borne gastroenteritis in humans, has a unique ability to colonize and replicate within chicken eggs which act as a main source of infection. SE ability to thrive under complex host environments is necessary to reach extra intestinal tissues such as liver, spleen and reproductive tract. Despite availability of whole genome sequences, molecular and genetic mechanisms underlying these characteristics are poorly understood. The objective of this study was to identify genes that are consistently highly expressed in multiple SE strains with high pathogenicities under different biologically relevant microenvironments encountered in the chicken host. Methods: Expression levels (High, Moderate and Low) of SE genes were predicted *in silico* using GEMBASSY-gphx. RNA-Seq was used for global transcriptome analysis of three highly pathogenic wild-type SE strains (UK, G1 and BC8) grown at avian body temperature (42°C) in two biologically relevant conditions that simulate intestinal (LB-Salt) or endosomal microenvironments (LMP broth). An artificial linear model was used to determine correlation between *in silico* gene expression levels (X) and RPKM values from *in vitro* transcriptome analysis (Y). Genes that were predicted to be highly expressed *in silico* and were consistently highly expressed across different microenvironments *in vitro* were manually searched, compiled into a database and classified according to Cluster of Orthologous Groups (COG). Results and Conclusions: A core of 23 genes are consistently highly expressed in three highly pathogenic *S. Enteritidis* strains, regardless of growth microenvironments representing the avian host. Most of these genes are highly conserved and non-essential, therefore can be deleted without significant effect on bacterial viability. Majority of genes are involved in outer membrane biogenesis and carbohydrate metabolism, and therefore may play a significant role in virulence of *S. Enteritidis*. Ongoing research aims to investigate the role of these genes in bacterial physiology and virulence, to identify candidates for development of new vaccines or antimicrobial agents.

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Identification and characterization of immune-modulatory CpG motifs of *Salmonella*

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Purpose: Cytine-guanine (CpG) motifs are DNA sequences (6 bp) that can have strong immune-modulatory activities. Certain motifs can elicit a protective response to pathogens such as *Salmonella*.

However, studies to identify the specific CpG motifs that are optimal in inducing a protective immune response have been limited. Our hypothesis is that specific CpG motifs within the *Salmonella* genome modulate the host immune response.

Methods: We identified 256 unique CpG motifs within the *Salmonella* pan-genome. We then synthesized 256 oligodinucleotides (ODNs) each containing a single CpG motif repeated 3 times. Previous studies have shown that this structure produces the optimal immunomodulatory response. We tested the immune-stimulatory properties of all ODNs using *in vitro* grown chicken macrophages. The induction of expression of IL-1 $\beta$ , a marker of immune stimulation, was determined for each ODN and ODNs were sorted by relative expression levels into 3 groups by K-means clustering: highly stimulatory, poorly stimulatory, and inhibitory. ODNs were selected from the highly stimulatory and inhibitory groups for secondary screening.

Results: Interestingly, 58 ODNs from the primary screen were found to induce IL-1 $\beta$  expression similar to the levels induced by both *Salmonella* lipopolysaccharide (a highly stimulatory membrane component) and a control ODN2007 (a well characterized stimulatory ODN). We confirmed that 37 of the ODNs identified in the primary screen were also stimulatory in the secondary screen. We are currently confirming the stimulatory properties of 37 ODNs.

Conclusions: In conclusion, this is the first study that comprehensively characterized the immune-modulatory activities of all the possible CpG motifs found within *Salmonella* pan-genome and identified a number of immunostimulatory motifs. These newly identified CpG motifs could serve as targets for the development of ODNs that stimulate immune responses against *Salmonella* in mammals and chickens, a reservoir host for *Salmonella* and also to improve the efficacy of currently used vaccines.

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Diversity and distribution of a novel swine dysentery pathogen “*Brachyspira hampsonii*”

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Outbreaks of bloody diarrhea in swine herds in recent years signaled the re-emergence of swine dysentery in North America caused by, not only *Brachyspira hyodysenteriae*, but also by a novel pathogen “*Brachyspira hampsonii*” 1. The pathogenicity of each distinct clade (I and II) of “*B. hampsonii*” has been confirmed experimentally in pigs2,3. The aim of this study was to study the diversity and distribution of “*B. hampsonii*” in the U.S.

A new multi-locus sequence typing (MLST) scheme based on six housekeeping genes of “*B. hampsonii*” was developed and used to characterize 64 “*B. hampsonii*” isolates obtained from the University of Minnesota Veterinary Diagnostic Laboratory in 2009-2014. Each isolate was characterized as a ‘sequence type’ (ST), closely related STs were grouped into ‘clonal complexes’ (CCs) and the STs were analyzed on three levels (intra-site, intra-system and inter-system).

The evaluated “*B. hampsonii*” isolates from the U.S. represented 11 STs (four of clade I and seven of clade II) and three CCs (one of clade I and two of clade II). No more than one ST was found in each site at a single point in time and, in general, different sites owned by a system shared one or more closely related STs. Sites and systems that were positive for “*B. hampsonii*” repeatedly over several years were found to be temporally infected with the same ST or two clonally related STs.

“*B. hampsonii*” genotypes were found to be epidemiologically related to their site and system of origin. Despite the more frequent isolation of “*B. hampsonii*” clade I in the U.S., this study demonstrates the higher genotypic diversity of “*B. hampsonii*” clade II. Analyses of other international isolates4,5 may provide a broader view of the diversity and distribution of “*B. hampsonii*” worldwide.

Acknowledgments

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The humoral immune response of pigs and horses against the vaccine of *Lawsonia intracellularis*.

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**Purpose:** *Lawsonia intracellularis* (Li) is the etiologic agent of porcine proliferative enteropathy and equine proliferative enteropathy. For the serological test, the indirect fluorescent antibody method (IFA) is often used. On the other hand, a live vaccine is already being used in Japan for the prevention of Li-infection in pigs. The vaccine still hasn't been approved for horses, but its efficacy has been shown in research. We investigated the humoral immune response of pigs and horses against Li-vaccine, and conducted a serological test against a vaccine-using horse farm to identify if epidemiological research is possible.

**Methods:** The Li-antibody after inoculation with Li-vaccine on piglets and foals was investigated. All serum samples were tested by the IFA. In trial one, ten piglets were allocated into 3 groups, then each group was inoculated with a 1×, 10× or 20× dose of the Li vaccine respectively, 2 times, 11 days apart. The sera were collected from each pig 3 times. In trial two, two foals were inoculated with the vaccine 2 times, 3 weeks apart, and serum was collected from each foal from 1 day before the first inoculation, to 5 weeks after the first inoculation. In trial 3, four foals from a horse breeding farm were inoculated with the vaccine, and we investigated the Li-antibody in the sera for 0 to 180 dpi.

**Results:** In trial one, Li-antibodies of all serum obtained from the inoculated piglets were negative. In trial 2, one foal had positive results throughout the entirety of the test. Another foal had a positive conversion 3 weeks after the first inoculation. In trial 3, the Li-antibody positive conversion was confirmed as 21 to 24 dpi in all foals, and the positive duration was between 35 to 134 dpi.

**Conclusions:** After the Li-vaccination, the Li-antibody in pig sera remained undetectable, meaning that it is possible to distinguish immune responses between Li-natural infections and Li-vaccinations. However, the Li-antibodies in the horse sera after inoculation with vaccine were detected, which indicated that combining several tests like PCR, not only serological tests, is necessary when conducting epidemiological research in horse farms that use vaccines.

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Temperature-dependent conjugative gene transfer in *Campylobacter jejuni*

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**Purpose:** Conjugation is an important mechanism for horizontal gene transfer in various niches for different foodborne pathogens, such as *Campylobacter jejuni*, the leading bacterial cause of human gastroenteritis in the United States. However, it has been observed that some *C. jejuni* strains, such as the widely used NCTC 11168, displayed extremely low conjugation efficiency. In this study, the effect of temporary high temperature treatment of *C. jejuni* recipient on conjugation efficiency was investigated.

**Methods:** The *Escherichia coli* DH5 $\alpha$  strain containing both helper plasmid RK212.2 and the shuttle vector pRY107 was constructed. Prior to be used for conjugation, *C. jejuni* cells were subjected to heat shock at different temperatures with variable length of incubation time. Several restriction-modification (RM) systems, such as Cj0030, Cj0139-40, Cj0690c and hsdR were inactivated to evaluate their roles in conjugation.

**Results:** Temporary high temperature treatment (50 °C) dramatically increased conjugation efficiency of NCTC 11168 and 81-176 for 10-100 folds. The filtrated supernatant from the heat shock-treated cells couldn't enhance the conjugation efficiency of non-heat shocked cells,

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suggesting the enhanced conjugation efficiency is independent of secreted substances. Mutation in different RM systems did not dramatically change conjugation efficiency of *C. jejuni*.

Conclusions: Heat shock treatment could dramatically increase conjugation efficiency of *C. jejuni*. This finding not only optimizes current conjugation protocol for effective molecular manipulation of *C. jejuni* but also facilitates us to better understand molecular basis of conjugation.

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Determination of the minimum infectious dose of porcine epidemic diarrhea virus in neonatal and weaned pigs

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Porcine epidemic diarrhea virus (PEDV) was first identified in the United States in April 2013 and rapidly spread to 30 states, suggesting that PEDV is highly transmissible. However, no information has been published regarding the minimum infectious dose (MID) of PEDV. In this study, serial ten-fold dilutions of a U.S. PEDV isolate resulting in theoretical infectious titers from  $5.6 \times 10^2$  to  $5.6 \times 10^{-4}$  TCID<sub>50</sub>/ml together with a negative cell culture medium control were prepared and orogastrically inoculated (10 ml/pig) into eight groups of 5-day-old pigs (n=4 per group; 4 pigs within each group were housed separately from each other) and 21-day-old pigs (n=6 per group; 6 pigs in each group were housed together), respectively. Rectal swabs were collected daily from neonatal pigs through 7 days post inoculation (DPI) followed by necropsy and from weaned pigs at 0-7, 10, 14, 21 and 28 DPI. Three weaned pigs per group were necropsied at 7 and 28 DPI, respectively. The infection status of pigs was determined by PCR on rectal swabs and cecal contents as well as microscopic evaluation and immunohistochemistry on small intestinal tissues. In 5-day-old pigs, a 10 ml inoculum of  $5.6 \times 10^2$  –  $5.6 \times 10^{-2}$  TCID<sub>50</sub>/ml (Ct values 24.2-37.6) resulted in 100% infection in each group while a 10 ml inoculum of  $5.6 \times 10^{-3}$  –  $5.6 \times 10^{-4}$  TCID<sub>50</sub>/ml (Ct>45) caused infection in 25% of the pigs. In 21-day-old pigs, a 10 ml inoculum of  $5.6 \times 10^2$  –  $5.6 \times 10^0$  TCID<sub>50</sub>/ml (Ct values 24.2-31.4) resulted in 100% infection in each group; however, a 10 ml inoculum of  $5.6 \times 10^{-1}$  –  $5.6 \times 10^{-4}$  TCID<sub>50</sub>/ml (Ct values 35.3->45) could not establish infection in any pigs. Our results confirmed that PEDV is highly infectious but in an age-dependent manner. The MID is significantly lower for neonatal pigs than for weaned pigs, which should be taken into consideration when interpreting clinical relevance of PEDV PCR results and when designing a PEDV bioassay model.

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Effect of pretreatment on detection of PRRSV in oral fluid by qRT-PCR assay

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Objective: Collection and testing of oral fluid samples is widely used for disease surveillance in swine. However, the oral fluid matrix is known to affect PCR performance, most often in the form of false negative results (1). Our objective was to evaluate the effect of specific factors on PRRSV qRT-PCR performance in the oral fluid matrix. Materials and Methods: 2.2 liters of oral fluid was collected from ~250 5-week-old pigs vaccinated 15 days prior with a MLV PRRSV vaccine. In the laboratory, the samples were pooled, stirred continuously while aliquoted into 25 ml volumes, and stored at -80°C. Factors evaluated in the study included: TEMP 1 (oral fluid thawed at 4°C or 25°C for 24 hours), DILUENT (1:2 dilution with either Trizol® or nuclease-free water; total volume = 1.5 ml), SONICATION for 10 minutes (yes/no), TEMP 2 (temperature at which sonication or no sonication was conducted; 4°C vs 25°C), and TEMP 3 (temperature at which the sample was held (4°C vs 25°C) until the PRRSV RT-qPCR assay was performed). Thereafter, samples were tested at ISU Veterinary Diagnostic Laboratory. In each replicate, 32 treatments were evaluated - 4 samples plus 1 negative control per treatment. 5 replicates were performed resulting in 800 PRRSV RT-qPCR results. Binary test outcomes were analyzed using logistic regression. All factors and their interactions were considered in the model. Insignificant effects were excluded from the final model. Results: Cumulatively, testing of 640 known positive samples produced 85 false negatives (86.7% sensitivity). Testing of 160 negative controls produced 1 false positive (99.4% specificity). Among positive samples, TEMP 1 significantly ( $p < 0.0001$ ) affected the outcome of a positive PCR. The odds ratio for the effect of TEMP 1 (25°C vs 4°C) was 0.265 (0.145, 0.447), i.e., a false negative result was far more likely when oral fluid was thawed at 25°C.

Conclusions: Pre-extraction factors associated with sample handling can significantly impact PRRSV qRT-PCR performance. Acknowledgments:

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Vaccination mitigates the negative impact PRRSV infection has on the pharmacokinetics of ceftiofur crystalline free acid in pigs.

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Purpose: The objective of this study was 1) Determine if PRRS modified live virus (MLV) alone impacts the pharmacokinetic (PK) profile of ceftiofur crystalline free acid in pigs, and 2) Determine if PRRS MLV vaccination prevents PK impacts when vaccinated pigs are challenged with a wild-type PRRS virus (PRRSv).

Methods: Thirty-eight high-health, PRRSV naïve barrows were allotted to one of four groups A) Control, n=8; B) Vaccinated Only, n=10; C) Challenged Only, n=10; D) Vaccinated Followed by Challenge, n=10. Respectively, on day -5 and -32, groups B and D were administered a 2mL intramuscular injection of Ingelvac® PRRS MLV vaccine (Boehringer Ingelheim Vetmedica). On day -5, groups C and D were intranasally challenged with PRRSV VR-2385. On day 0, all groups were intramuscularly administered ceftiofur crystalline free acid (CCFA, Excede for Swine® Zoetis) at a 5 mg/kg rate. Blood was sampled 0 (pre-treatment), 0.25, 0.5, 1, 6, 12, 24, 48, 96, 144, 192, and 240 hours post-injection. Plasma was analyzed for ceftiofur and related metabolites using liquid chromatography coupled with mass spectrometry. Non-compartmental PK modeling (WinNonlin 5.2) determined: area under curve time 0 to last time point (AUClast), maximum concentration (Cmax), time to maximum concentration (Tmax), terminal half-life ( $T_{1/2\lambda z}$ ), clearance per fraction of dose absorbed (Cl/F), volume of distribution per fraction of dose absorbed (Vz/F). Mixed model ANOVA was used to compare PK parameters between groups using Tukey's test to determine significant mean

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differences,  $P \leq 0.05$  (SAS 9.3).

Results: Group A and Group B were not significantly different on any evaluated PK parameter. When compared to Group C, Group D had significantly higher AUClast, lower C<sub>1</sub>/F, shorter T<sub>1/2</sub> λ<sub>z</sub>, and smaller V<sub>z</sub>/F.

Conclusions: Vaccination with Ingelvac® PRRS MLV does not influence PK parameters of CCFA. When challenged, vaccination, allowed more CCFA in the vasculature, indicated by a larger AUClast. Terminal half-life was shorter in pigs that were vaccinated before challenge and could reflect changes in bioavailability, which was not measured in this study, due to infection.

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Genetic diversity analysis of genotype 2 porcine reproductive and respiratory syndrome viruses emerging in recent years in China

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Purpose: Porcine reproductive and respiratory syndrome virus (PRRSV) is characterized by its extensive genetic diversity in the field. The aim of this study is to investigate the genetic diversity of PRRSV emerging in recent years in China.

Methods: Here we gained 101 sequences of NSP2 hypervariable region, 123 ORF3 gene sequences and 118 ORF5 gene sequences from 128 PRRSV-positive clinical samples collected in different areas of China during 2008-early 2012.

Results: Comparative analyses of these sequences indicated that the amino acid identities of the three genes among these sequences were 87.6%-100%, 92.5%-100% and 77%-100% respectively. Meanwhile, 3 novel patterns of deletion and insertion in NSP2 region, and an amino acid deletion in GP5 were first found. The phylogenetic analysis based on NSP2, ORF3 and ORF5 genes revealed that the Chinese PRRSV strains could be divided into three subgroups; majority of genes analyzed in this study were clustered in the subgroup 3 with multiple branches; the strains with 30-aa deletion in NSP2-coding region were still the dominant virus in the field. Furthermore, four isolates were subjected to full-length genomic sequencing, and the phylogenetic analysis based on the complete genomic sequences showed they were clustered into different branch with the Chinese corresponding representative strains.

Conclusions: Our analyses suggest that the genetic diversity of genotype 2 PRRSV in the field displays a tendency of increasing in recent years in China, and the 30-aa deletion in NSP2-coding region should be no longer defined as the molecular marker of the Chinese HP-PRRSV.

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*In vivo* targeting of porcine reproductive and respiratory syndrome virus antigen through porcine DC-SIGN to dendritic cells elicits antigen-specific CD4 T cell immunity in pigs

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Immunogenicity of protein subunit vaccines may be dramatically improved by targeting them through antibodies specific to c-type lectin receptors (CLRs) of dendritic cells in mice, cattle, and primates. This novel vaccine development approach has not yet been explored in pigs or other species largely due to the lack of key reagents. In this study, we demonstrate that porcine reproductive and respiratory syndrome virus (PRRSV) antigen was targeted efficiently through antibodies specific to a porcine CLR molecule DC-SIGN (dendritic cell-specific intercellular adhesion molecule-3-grabbing non-integrin) in pigs. A recombinant PRRSV antigen (shGP45M) was constructed by fusing secretory-competent subunits of GP4, GP5 and M proteins derived from genetically-shuffled strains of PRRSV. In vaccinated pigs, when the PRRSV shGP45M antigen was delivered through a recombinant mouse-porcine chimeric antibody specific to the porcine DC-SIGN (pDC-SIGN) neck domain, porcine dendritic cells rapidly internalized them *in vitro* and induced higher numbers of antigen-specific interferon-γ producing CD4 T cells compared to the pigs receiving non-targeted PRRSV shGP45M antigen. The pDC-SIGN targeting of recombinant antigen subunits may serve as an alternative or complementary strategy to existing vaccines to improve protective immunity against PRRSV by inducing efficient T cell responses.

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Neuraminidase inhibiting (NI) antibodies induced in pigs by experimental influenza A virus vaccines

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The neuraminidase (NA) protein of influenza A viruses (IAV) has important functional roles in the viral replication cycle. Antibodies specific to NA can reduce viral replication and limit disease severity. Analysis of NA inhibiting (NI) antibodies in vaccinated or infected animals was often bypassed because of the cumbersome traditional assay. In recent years a simpler method, called the enzyme-linked lectin assay (ELLA), has been utilized to quantify serum NI antibodies. We aimed to analyze systemic and respiratory mucosal NI antibodies in piglets with actively or passively acquired immunity to IAV.

Using the 96-well ELLA protocol we quantified NA-mediated sialic acid removal from plate-bound fetuin in the presence or absence of antibodies. We tested serum and respiratory specimens from a study comparing whole-inactivated virus (WIV) vaccine, intranasal live-attenuated influenza virus (LAIV), and intranasal wild type (WT) IAV infection. Additional sera were from a study investigating maternally-derived antibodies (MDA) and effects of passive immunity on vaccination. Test antigens were reassortant viruses containing NA genes homologous or heterologous to the vaccines.

Naïve piglets responded to WIV and LAIV vaccines and WT infection with strong homologous serum NI titers. Cross-reactivity to heterologous NAs depended on the degree of genetic divergence. Bronchoalveolar lavage specimens of LAIV and WT-immunized groups had significant NI activity to the homologous antigen. Piglets of vaccinated sows received high levels of passive NI antibody, but their NI responses to homologous LAIV vaccination were impeded.

Although often overlooked, NA antigen has a significant role in IAV immunity and vaccine composition. We have demonstrated the utility of the ELLA format for efficient NI antibody titration of serum and respiratory tract secretions from pigs. Swine IAV vaccines that induce robust NI responses are likely to provide broader protection against the diverse and rapidly evolving IAV strains that circulate in pig populations.

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Influenza transmission within coordinated swine production systems

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Influenza viruses are a common cause of respiratory disease in swine. Multiple serotypes of influenza A (INFA) infect pig populations. We have demonstrated that it is routine to identify INFA in pigs without appreciable clinical disease. Little is known about the routes of transmission of INFA between pig populations; specifically what are the most common sources of novel INFA genotypes. We report the preliminary results from four closed, multi-site swine production systems that were sampled for 21 consecutive months across all phases of genetic multiplication and commercial weaned pig production. Each production system consisted of six sites: One for the breeding and farrowing of genetic replacement animals (MF), one for growing replacement gilts (GDU), and four for breeding and farrowing commercial sows (BTW). MF farm made their own replacement gilts with only semen being introduced. In two systems MF replacement animals were raised on site and two systems they were raised at the GDU with the replacement females for the BTW farms. Thirty nasal swabs were collected each month from the following groups of animals: 7-8 month at MF (GMF), 21 day old at MF (WMF), 5-6 month old at GDU (SEL), 7-8 month old at BTW (GBTW), and 21 day old at BTW (WBTW). All samples were evaluated using rrtPCR. Positive samples were subjected to HA and NA typing and full genome sequencing. Results from 23429 samples collected between October 2012 and June 2014 are reported. INFA was detected in 83/84 sample periods across all four systems, in 55% of the 783 sample sets ( $\geq 1$  positive/30 swabs) and in 7.9% of samples. Sample set prevalence was 60% GMF, 62% WMF, 59% SEL, 49% GBTW and 59% WBTW. Multiple HA and NA types were detected in each of the systems across time and in specific sample sets. Sequencing will further elucidate the role of lateral and vertical transmission of INFA in modern production systems. These data are the first to describe the prevalence and serotype of INFA in coordinated swine production systems over time. The majority of farms are infected with INFA on a continuous basis. Multiple reservoirs of INFA in breeding herds and production systems are likely based on nearly equal prevalence across multiple ages of pigs.

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Co-circulation of two distinct genetic and antigenic lineages of proposed influenza D virus in cattle

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Viruses with approximately 50% homology to human influenza C virus (ICV) have recently been isolated from swine and cattle. The overall low homology to ICV, lack of antibody cross reactivity to ICV in hemagglutination inhibition (HI) assay and agar gel immunodiffusion assays and inability to productively reassort with ICV led to the proposal that these viruses represented a new genus of influenza, influenzavirus D (IDV). Previous serological surveys found 88% of bovines had geometric mean titers greater than 40 in the HI assay. To further our understanding of the epidemiology of IDV, real time reverse transcription PCR was performed on a set of 208 samples from bovines with respiratory disease. Ten samples (4.8%) were positive and successfully propagated in vitro. Phylogenetic analysis of ten full genome sequences revealed two distinct co-circulating lineages represented by D/swine/Oklahoma/1334/2011 (D/OK) and D/bovine/Oklahoma/660/2013 (D/660) which frequently reassort with one another. Antigenic analysis using the HI assay and D/OK and D/660 antiserum found up to an approximate 10-fold loss in cross reactivity using heterologous clade antiserum. One isolate, D/bovine/Kansas/3-13/2011 (D/3-13), had higher HI titers to heterologous clade antiserum. Genetic analysis and molecular modeling of the predicted hemagglutinin esterase fusion amino acid sequence of D/3-13 identified amino acid R212 as a possible antigenic determinant responsible for the discrepant HI results. These results suggest that IDV is commonly found in bovines with respiratory disease and at least two genetic and antigenically distinct clades co-circulate.

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An RSV fusion inhibitor is an effective treatment for bovine respiratory syncytial virus infection of calves

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Bovine respiratory syncytial virus (bRSV) causes severe lung disease in young calves. Human infants infected with human RSV develop a very similar respiratory disease, which can result in fatalities. A new antiviral compound targeting the fusion protein of human RSV was tested in the bovine as an experimental infection model. In vitro testing demonstrated potent antiviral activity against bRSV. Pharmacokinetic evaluation in calves following intravenous administration demonstrated acceptable plasma and lung tissue exposures. The ability of the compound to suppress viral replication, diminish clinical signs, and decrease lung pathology was then examined in two studies in young calves experimentally infected with bRSV. In the first study the compound was administered twice daily beginning 24 hours after bRSV infection. In the second study a compound was administered once-daily beginning at 24 hours or 72 hours after viral infection. Both studies compared treatment with the antiviral compound and placebo. Viral shedding was measured by qRT-PCR from nasal swabs and lung lavage fluid. A blinded investigator scored clinical signs daily; lung pathology was evaluated by a blinded board certified veterinary pathologist. In the first study treatment with the antiviral compound reduced the viral load in nasal swab samples by approximately 95% compared to the placebo treated group. Clinical scores were significantly lower in treated calves when compared with placebo. Lung consolidation, presence of bronchiolitis obliterans and bronchointerstitial pneumonia were prominent features in the placebo group but not in the calves treated with antiviral compound. Both treatment regimens were effective, but the treatment initiated 24 hours after infection resulted in a more significant therapeutic effect. In summary, a RSV fusion protein inhibitor demonstrated an antiviral and clinical effect in the bovine experimental model supporting the potential for the treatment of bRSV infection in calves. In addition, the bRSV experimental infection may serve as a relevant in vivo preclinical efficacy model to assess novel anti-RSV therapeutic strategies.

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Improving case definitions for BRD treatment with an algorithm based analysis of lung auscultation

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Bovine Respiratory Disease (BRD) is the primary cause of morbidity and mortality in cattle during the feeding period. Management of BRD cases relies on the observation of cattle behavioral changes by caregivers. After identification, sick cattle are typically moved to a treatment area, where based on rectal temperature (RT), attitude and appearance, a case definition (CD) for BRD presence, severity and chronicity is assigned by a trained caregiver. Sometimes manual thoracic auscultation is used to refine CD. The implementation of evidence based therapies, however, is ineffective as this process, with or without manual auscultation, is highly variable within and between caregivers. We applied a computer based algorithm (Whisper®, Geissler Corp.<sup>2</sup>) to sounds recorded during the initial assessment for suspected BRD of 17,848 cattle from 13 sites to determine if algorithm based analysis of lung auscultation would improve BRD CD precision. Briefly, we placed the microphone on the right side of the thorax per manufacturer's instructions to capture 8 seconds of sounds that were processed with the software. A discrete score (LS) of 1 to 5 was applied to each calf (1= normal, 5= chronic - severe BRD) for use in the treatment decision protocol. RT was also a factor in the treatment protocol. Outcomes were measured by case fatalities (CF) - individuals that died of respiratory disease following treatment protocol. We expected a high LS to be associated with a greater chance of CF. A logistic regression model using BW, RT and LS was constructed to estimate the likelihood of CF. For each 1 unit rise in LS the likelihood of CF increased by 49.4% adjusted for RT and BW. Odds ratio (OR) = 1.494 (95% CI: 1.428, 1.564). For each 1 degree F increase in rectal temp the likelihood of CF increased by 19.1%. OR = 1.191 (95% CI: 1.149, 1.234). There was a statically significant but biologically insignificant impact of BW on CF. These data suggest that algorithm based analysis of lung auscultation is an effective tool under real world conditions to predict clinical outcomes. It can be applied to implement standard case definitions for BRD to assist the development of evidence based treatments for BRD.

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Identification of pens at high risk for BRD with an algorithm based analysis of thoracic auscultation at post arrival processing

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Bovine Respiratory Disease (BRD) is the primary cause of morbidity and mortality in cattle during the feeding period. BRD is commonly diagnosed in the first 21 days post entry to the feedlot. In cattle perceived to have a high risk of developing BRD it is common to apply prophylactic antibiotic therapy (PA) at the time of arrival. The ability to predict if a group will have a high rate of BRD post arrival is imprecise at best, making evidence based application of prophylactic antibiotics impossible. Objective tools to assess the health of the respiratory tract at the time of feedlot arrival would allow for the application of PA in only groups of cattle with a significantly increased risk of developing BRD. We applied a computer based algorithm (Whisper®, Geissler Corp.2) to sounds from thoracic auscultation recorded during post arrival processing of 25 groups containing 2069 cattle (X=83/pen, 95% CI 64-101) in one feed yard to determine if algorithm based analysis of lung auscultation at arrival would facilitate the identification of pen-lots with higher BRD morbidity over the first 30 days post placement. Briefly, we placed the microphone on the right side of the thorax per manufacturer's instructions to capture 8 seconds of sound that were processed with the software. A discrete score (LS) of 1 to 5 was applied to each calf (1= normal, 5= chronic - severe BRD). Rectal Temperature (RT) and Body weight (BW) were also recorded. Outcomes were measured as BRD morbidity in the first 30 days post placement. Cattle with a LS>=3 or RT>=104.5°F (40.3°C) were treated with antibiotics. Stepwise linear regression was used to determine the value of algorithm-based auscultation at arrival as a predictor of group BRD morbidity rate. The final model was [% Morbidity = 0.65 x % LS 3 at arrival] (R2=0.62, p<0.001) meaning that a 1% increase in LS3 prevalence at placement was associated with a 0.65% increase in morbidity even with treatment of diagnostically abnormal cattle at arrival. These data suggest that algorithm based analysis of thoracic auscultation is an effective tool under real world conditions to predict groups at risk of high BRD morbidity. Further research is needed to adapt PA protocols in an evidence based manner.

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Acute phase proteins in naturally occurring respiratory disease of feedlot cattle: a novel approach to diagnosis.

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Bovine respiratory disease (BRD) is the most costly disease of feedlot cattle in the United States. Costs associated with BRD prevention, treatment, morbidity, and mortality have been estimated from \$13.90 to \$15.57 per head with annual losses to the cattle industry exceeding \$750 million. The development of BRD involves a variety of anatomical, physiological and management factors which increase susceptibility to viral and bacterial pathogens. A presumptive diagnosis of BRD is usually based on clinical signs including elevated rectal temperatures. Physical exam alone lacks high sensitivity and specificity, leading to misclassification and unnecessary treatment or failure to treat true cases. More sophisticated diagnostic tests exist but are not practical in feedlot settings. The objective of this study was to evaluate the utility of three acute phase proteins as a method of improving BRD diagnosis in conjunction with a respiratory scoring system. A study population of 77 beef calves was observed for signs of BRD. In total, 14 cases and pen matched controls were included in the initial data analysis. The results indicated a significant increase in BRD scores, lipopolysaccharide binding protein (LBP), haptoglobin and decrease in total feed intakes (P < 0.01 from T Test) in the respiratory cases versus controls. Monitoring LBP, haptoglobin levels along with performance variables, may be useful as prognostic tools and facilitate treatment decisions.

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Cytokine profiles from shipping through sickness and recovery in cattle either mass-medicated with gamithromycin or sham-treated

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Bovine Respiratory Disease (BRD) is estimated to be responsible for 75% of morbidity and 50% of mortality in beef cattle feeding operations, and mass medication of cattle upon arrival at feeding facilities is a common practice to control BRD. Our objectives were to determine if cytokine profiles differed between sham- and mass-medicated treatment groups, and if cytokine profiles differed between animals presenting with BRD and those that remained healthy throughout a 28 day trial regardless of antibiotic use. Sixty head of cattle were purchased at each of three sale barns located in MO, TN, and KY for a total of 180 animals on trial. Cattle were transported to a feeding facility in KS where they were randomly allocated within source to one of two treatments, mass-medication with gamithromycin (n = 90) or sham saline-injection treatment (n = 90).

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Blood samples were collected for plasma at Day 0 (at sale barn), Day 1 (at KS facility), Day 9, and Day 28. Cattle presenting with BRD were also sampled for plasma at the time of diagnosis and 5 days later. The plasma samples were assayed for the cytokines IL-1 $\beta$ , IL-4, IL-6, TNF- $\alpha$ , and IFN- $\gamma$  using a multiplexed electrochemiluminescent assay system. BRD cases were evenly distributed across sale barns (9, 9, and 10). Nineteen sham-saline treated animals presented with BRDC as compared to 9 animals in the mass-medicated group. Differences were observed in the cytokine profiles between the mass-medicated and sham-saline treated groups. Preliminary analyses indicate that mass-medication with gamithromycin resulted in increased production of IL-1 $\beta$ , IL-4, IL-6, and IFN- $\gamma$  in animals that presented with BRD as compared to sham-saline treated animals that presented with BRD. However, TNF- $\alpha$  production did not appear to vary either between the mass-medicated and sham-saline treated groups, or between animals that remained healthy or presented with BRD. Additional analysis is on-going. USDA is an equal opportunity employer and provider.

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Identification and characterization of bovine rhinitis viruses in bovine respiratory disease clinical specimens

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Recently, the three species of bovine rhinovirus were reclassified as two species of bovine rhinitis virus (BRV), with the former bovine rhinovirus 1 and 3 designated as serotypes of bovine rhinitis A virus (BRAV-1 and BRAV-2, respectively) while bovine rhinovirus 2 was reclassified as bovine rhinitis B virus. Despite their isolation from diseased calves and ability to cause disease in naïve animals, few studies have been performed investigating the role of these viruses in bovine respiratory disease (BRD). Viral metagenomic sequencing was performed on a nasal swab submitted for diagnostic testing. Besides recovering a near complete bovine coronavirus genome, numerous contigs showed high homology to BRAV-2. Templated assembly with the sole BRAV-2 reference in GenBank gave a near-complete genome spanning 7,175 bp with 89% identity to BRAV-2 strain H-1 originally isolated in Japan in 1985. A Taqman assay designed using BRV sequence from the nasal swab was used to screen a collection of 204 BRD samples submitted to KSVDL in 2013-2014. Thirteen of 204 samples (6.4%) were positive with Ct values ranging from 17.9-29.4. Likewise, the presence of other bovine respiratory viruses was determined by PCR. The most commonly identified virus co-infecting animals with BRV was a recently proposed influenza D virus, followed by bovine respiratory syncytial virus, bovine coronavirus, bovine viral diarrhoea virus and infectious bovine rhinotracheitis. Further viral metagenomic sequencing of BRV-positive nasal swabs yielded six partial genomes. Two samples had 86-89% identity to BRBV while four had high identity (87-89%) to BRAV-2, indicating that the Taqman assay detects both species of BRV and that both species circulate in U.S. bovine herds. Further research is needed to determine a possible etiologic role for BRAV and BRBV in BRD.

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Characterization of Biofilm Formation by *Pasteurella multocida*

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**Purpose:** *Pasteurella multocida* is an important zoonotic agent responsible for a wide range of animal diseases, including bovine respiratory disease, atrophic rhinitis, and fowl cholera. The ability of *P. multocida* to cause infection has been attributed to its wide range of virulence factors. We believe that biofilm formation by *P. multocida* may be essential to establishing a persistent infection, which is common in the carrier state of migratory birds and transported cattle.

**Methods:** Isolates from avian and bovine were screened for their capability to form biofilms. The bacteria were grown to stationary phase in RPMI-1640 medium at 37 °C for 1-3 days with or without the addition of hyaluronidase or capsular material. Biofilms were quantified by crystal violet staining, protein, and polysaccharide assays, and observed by scanning electron microscopy (SEM), and confocal scanning laser microscopy (CSLM). A novel exopolysaccharide (EPS) produced during biofilm formation was purified by phenol extraction, enzyme digestion, and gel filtration. The EPS was hydrolyzed, derivatized, and analyzed using <sup>1</sup>H NMR and gas chromatography-mass spectrometry. Gene clusters of putative significance for EPS production are being tested by quantitative reverse transcriptase-polymerase chain reaction. One such cluster includes homologs of the genes important for *Histophilus somni* EPS production: *galU*, *manB*, and *csrA*.

**Results:** Acapsular mutants containing a disrupted *hyaE* gene and wildtype strains that produced less capsule, as determined by Congo red staining, CLSM, and SEM, formed more biofilm and EPS than isolates that produced more capsule. The EPS was composed primarily of mannose and galactose. Addition of hyaluronidase to the growth medium increased biofilm formation. However, the addition of capsular material (hyaluronic acid) did not inhibit biofilm formation.

**Conclusions:** We have confirmed that *P. multocida* forms a biofilm *in vitro*, and that biofilm production is inversely related, but not necessarily inhibited by, capsule production. Future *in vivo* studies will provide valuable insight into the importance of biofilm formation on virulence.

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Septic pleuropneumonia in 41 horses (2000 - 2014)

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**Purpose:** To investigate signalment, history, physical examination findings, clinicopathological and bacteriological data, treatments and outcome in horses diagnosed with septic pleuropneumonia.

**Methods:** A retrospective study was performed. Horses were included in the study if they were diagnosed with sepsis (based on criteria for SIRS and confirmed infection) at admission, and demonstrated pleural effusion via transthoracic ultrasonography. The study period was from 2000 to 2014.

**Results:** Forty-one horses met the inclusion criteria. The median age was 4 years, and travel within 6 weeks of admission was the most common risk factor. Common historical complaints included fever (68% of cases), inappetence (63%) and lethargy (59%), while common physical examination findings at admission included tachycardia (85%), tachypnea (80%) and fever (39%). Hyperfibrinogenemia and band neutrophilia were present in 90% and 73% of cases, respectively. Other common clinicopathological abnormalities included hyperglobulinemia (69%),



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hyponatremia (67%), and hyperbilirubinemia (54%). The most common bacteria isolated from tracheal wash and pleural fluid was *Streptococcus equi* subsp. *zooeidemicus* (65%). Within individual animals, only 21% of tracheal wash and pleural fluid samples yielded the same results. The most common antimicrobial regimen included penicillin, gentamicin and metronidazole. Laminitis was diagnosed in 15% of horses. A thoracotomy was performed in 9 horses (22%). The median duration of hospitalization was 11 days. The survival rate to discharge from the hospital was 59%.

Conclusions: Racehorses with a recent history of traveling are at risk of developing septic pleuropneumonia. Horses typically present with severe inflammation, as reflected by physical examination and blood work findings. Upper respiratory tract flora continues to represent the most common bacteria isolated from the lower airway of horses with pleuropneumonia. Broad spectrum antimicrobials and removal of septic thoracic fluid are critical components of treatment. With aggressive therapy, the prognosis for horses with septic pleuropneumonia is fair.

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Regulation of interferon-gamma gene expression in foals and its relationship to susceptibility to *Rhodococcus equi*.

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*Rhodococcus equi* remains the most common cause of bronchopneumonia in foals and continues to have a major financial impact on equine agribusiness. While the pathogenesis of *R. equi* in foals is becoming better understood, current control strategies for preventing *R. equi* infections are expensive, inconvenient, and not always effective. Though significant efforts have been expended on identifying protective antigens, there is currently no vaccine available for the prevention of *R. equi* pneumonia in foals despite considerable work using murine and equine models. Indeed, since exposure of foals to *R. equi* likely occurs within the first days of life, the development of an effective vaccine that prevents *R. equi* infections may prove difficult. Nevertheless, some optimism may be garnered from studies indicating older foals and adult horses are resistant to this infection. Additional information regarding the underlying mechanism of this resistance is needed before vaccination or other control strategies can be developed.

Our underlying hypothesis is that susceptibility to *R. equi* is regulated at two points in the young foal's life. Initial susceptibility to foals is associated with a likely failure of an innate immune mechanism. This susceptibility extends through the first month of the foal's life and is followed by the acquisition of almost complete resistance to infection. This period of susceptibility is temporally associated with decreased expression of interferon-gamma (IFN- $\gamma$ ) due to epigenetic modification of the *ifng* locus. The second resistance mechanism occurs post-infection when most foals spontaneously resolve their lesions. This resistance likely involves an adaptive immune response directed against the bacterium. This talk will highlight the work that my group has done characterizing these resistance mechanisms.

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Real-time PCR Assay Validation for Detecting *Rickettsia rickettsii* infections in dogs and ticks

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Rocky Mountain spotted fever (RMSF) is a disease of people and dogs caused by the rickettsial pathogen, *Rickettsia rickettsii*. *R. rickettsii* and closely related members of the spotted fever group of *Rickettsia* are considered endemic throughout North and Central America. These pathogens are transmitted primarily through the bites of infected ticks. In the USA, *Dermacentor variabilis* (the American dog tick) and *D. andersoni* (the Rocky Mountain wood tick) are considered as the primary vectors for transmitting *R. rickettsii*. A real-time polymerase chain reaction (PCR) assay described recently for human sample analysis (Kato et al., 2013) was re-evaluated for its use in detecting *R. rickettsii* infections in canine blood samples and ticks. A recombinant plasmid containing the target gene segment was prepared and serial dilutions of the plasmid were used for defining the analytical sensitivity of the assay; an equivalent to 1 copy plasmid was detected. For analytical specificity, DNA samples from other tick borne pathogens of dogs were assessed and the assay showed no amplification with genomic DNAs of *Ehrlichia chaffeensis*, *E. canis*, *Anaplasma phagocytophilum*, *A. platys*, and *Borrelia burgdorferii*. To validate the assay for clinical samples, genomic DNAs isolated from plasma of 97 clinically suspected dog blood samples were evaluated. Sixteen of these DNAs tested positive by the real-time PCR assay. The positives were further confirmed by a nested PCR assay targeting to an outer membrane protein gene of the pathogen, namely *Omp A* which is also conserved in other spotted fever group *Rickettsia*. Twelve out of 16 samples tested positive by nested PCR. Further, the specificity of the amplicons was confirmed by sequence analysis. A total of 222 *D. variabilis* ticks collected from the state of Kansas were subjected to the real-time PCR analysis. Fourteen of the ticks tested positive (6.3% of ticks) for *R. rickettsii* DNA. The DNA from the positive ticks was further subjected to the nested PCR and sequence analysis. Three samples were further confirmed by the nested PCR assay. Together, the data demonstrate that the real time PCR assay is useful for diagnosing *R. rickettsii* infections in both dogs and ticks.

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Molecular prevalence of *Theileria* spp. in ruminants from nine provinces of China

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*Theileria* spp. are tick transmitted protozoa that can infect large and small ruminants causing disease and economic losses. Diagnosis of infections is often challenging as parasites can be difficult to detect and identify microscopically and serology is unreliable. While there are PCR assays which can identify certain *Theileria* spp. there is no one PCR that has been designed to identify all recognized species that occur in ruminants and which will greatly simplify the laboratory diagnoses of infections.

Primers and probes for a genus-specific pan-*Theileria* FRET-qPCR were selected by comparing sequences of recognized *Theileria* spp. on GenBank and the test validated using reference organisms. The assay was also tested on whole blood samples from large and small ruminants from nine provinces in China.

The pan-*Theileria* FRET-qPCR detected all recognized species but none of the closely related protozoa. On whole blood samples from animals in China, *Theileria* spp. DNA was detected in 53.2% of the sheep tested (59/111) and in 44.4% of the goats (120/270) and 30.8% of the cattle (380/1,235). Water buffaloes (n=29) were negative. Sequencing of some of the PCR products showed cattle in China were infected with *T. orientalis*/

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*T. sergenti*/*T. buffeli* group while *T. ovis* and *T. luwenshuni* were found in sheep and *T. luwenshuni* in goats. The prevalence of *Theileria* DNA was significantly higher in *Bos p. indicus* than in *Bos p. taurus* (77.7 % vs. 18.3 %) and copy numbers were also significantly higher (104.88 vs. 103.00 *Theileria* 18SrRNA gene copies/per ml whole blood).

The pan-*Theileria* FRET-qPCR can detect all recognized *Theileria* spp. of ruminants in a single reaction and sequencing of products can identify the species. Large and small ruminants in China are commonly infected with a variety of *Theileria* spp.

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Portable insulated isothermal RT-PCR (iiRT-PCR) assay for sensitive and specific detection of bluetongue virus

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Bluetongue is a non-contagious hemorrhagic disease of ruminants transmitted by *Culicoides* midges. The disease is endemic in many tropical, subtropical and temperate regions, including Africa, Southern Asia, Northern Australia and the Americas. The causative agent, bluetongue virus (BTV), is a member of the *Orbivirus* genus of the *Reoviridae* family. Twenty six BTV serotypes have been identified throughout the world and five of them are enzootic in the USA, and ten additional exotic BTV serotypes have been isolated from Southern US. Canada is considered BTV free except in the Okanagan valley in BC. Based on previous and on-going serological testing, BTV incursions continue to occur in the Okanagan valley and the surrounding region. Many laboratory-based molecular assays for detection of BTV have been developed. These methods require the samples to be shipped to central laboratories, and highly skilled technicians and sophisticated instruments to perform the assays and interpret the results.

Portable user-friendly on-site molecular diagnostic assays overcome these impediments. Here we report the development and evaluation of a portable insulated isothermal RT-PCR (iiRT-PCR) assay for sensitive and specific detection of BTV. The BTV iiRT-PCR assay based on BTV NS1 gene detects all twenty six BTV serotypes and does not cross react with epizootic hemorrhagic disease virus (EHDV), a closely related *Orbivirus* of ruminants. The assay detects as low as 9 copies of *in vitro* transcribed BTV RNA with a 95% probability. RNA extracted from blood samples collected from experimentally BTV infected sheep and cattle shows that the BTV iiRT-PCR assay is as sensitive as the BTV real-time RT-PCR assay currently being used at the CFIA-National Centre for Foreign Animal Disease, Winnipeg, MB. This novel, highly-sensitive, low-cost and rapid BTV molecular assay can be performed and interpreted within one and half hours at farm-sites by a veterinarian without prior knowledge of molecular biology.

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Surveillance of ecto- and endoparasitism in northern Mississippi canine shelter populations

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Parasitism of companion animals not only affects animal health and wellbeing but also has public health and zoonotic ramifications. Additionally, in shelter populations, high parasite burden can cause animals to be anemic, immune-suppressed, poor surgical candidates for spay/neuter, and poor responders to vaccination. The purpose of this study was to evaluate the prevalence of parasitism in northern Mississippi shelter canine populations utilizing data collected during the training of junior veterinary students. All canines involved in the study were selected by shelter personnel for veterinary examination as part of the intake process or for a specific health concern. Examinations were performed by students under the direct supervision of a veterinarian. Skin scrapes were performed when alopecia with crusting or scale was present. Simple fecal flotation was performed with sodium nitrate solution (Fecasol). Fifty-three physical exams with fecal floats were performed at three northern Mississippi shelters on canines in the course of 12 weeks. 62% of canines had one or more ectoparasites present (45% having *Ctenocephalides* spp., 34% various tick spp., 9% *Demodex* spp., and 2% *Otodectes* spp.). 60% had one or more endoparasites present (36% having *Ancylostoma* spp. oocytes, 34% *Toxocara* spp. oocytes, 13.2% *Isospora* spp., 8% *Trichuris* spp. oocytes, 3.8% *Dipylidium* or *Taenia* spp. oocytes or segments, and 1.9% *Physaloptera* spp. oocytes). 28.3% dogs had 2 or more internal parasites present and 24.5% had 2 or more external parasites present. 50.9% of dogs had 2 or more total parasites present on examination. Preliminary results demonstrate that the majority of dogs entering shelters in north Mississippi have at least one form of parasitism stressing the importance of parasite control in shelter canine populations.

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Evaluation of *Dermacentor variabilis* for the incidence of pathogens causing diseases in animals and humans

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There is an emerging interest in the surveillance of tick borne diseases due to their increased incidence during the past several decades. Ticks are the second most important vectors for causing illnesses in animals and people. They transmit the largest variety of pathogens, including parasites, bacteria, and viruses. In this study, we evaluated *Dermacentor variabilis* ticks for the presence of three bacterial pathogens for which it is considered as the transmitting vector; *Anaplasma marginale*, *Francisella tularensis* and *Rickettsia rickettsii*. Often known as the American dog tick, *D. variabilis* is a 3-host tick that is prevalent in the eastern United States and can feed on both small and large mammals, including humans. *A. marginale* is the causative agent for bovine anaplasmosis; *F. tularensis* is responsible for tularemia in small animals and humans; while *R. rickettsii* causes Rocky Mountain Spotted fever, primarily in dogs and people. A total of 222 *D. variabilis* ticks were collected in Kansas to evaluate the incidence of the three pathogens, including single infection as well as co-infections. The analysis was performed by conventional (*A. marginale* and *F. tularensis*) and real-time (*R. rickettsii*) polymerase chain reaction assays. The incidence of *A. marginale* was higher at 33% (73 ticks), followed by *F. tularensis* (18%; 40 ticks), and *R. rickettsii* (6%; 14 ticks). The incidence of co-infection for *A. marginale* and *F. tularensis* was 12% (26 ticks) and for *A. marginale* and *R. rickettsii* was 2% (4 ticks). There were no ticks that tested positive for co-infection with *F. tularensis* and *R. rickettsii*. These data demonstrating a higher prevalence of the pathogens highlights the significant concern for health and wellbeing of both human and animals.

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Investigation into horn fly burden susceptibility in Holstein heifers

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Horn flies (*Haematobia irritans*) are economically important parasites of cattle. As fly burdens increase, animal performance decreases due to annoyance, increased disease and decreased feed conversion efficiency. Many insecticide products are losing efficacy, prompting research into alternative control methods. In this study, conducted over the summer of 2014, a herd of Holstein heifers, untreated with insecticides, was used to investigate whether a single determinant could be found to explain differences in fly burdens between individuals. Thirty heifers were randomly selected from the herd at two separate time intervals, six weeks apart. Heifers were housed on the same pasture and fed the same throughout. All heifers were photographed in a standardized fashion, and fly counts per side were made from digital images. From the group of 30, 10 animals were selected based on fly counts. The five most highly burdened (HB) animals were compared to the five with lowest burdens (LB). During the first trial period the average fly count of the HB was 5.68 times greater (151.6+/-41.4 vs. 26.7+/-2.4,  $p=0.0001$ ) when compared to LB heifers. For the second trial period the HB heifers had 6.01 times (236.9+/-40.6 vs. 39.4+/-6.2,  $p<0.0001$ ) more flies than LB heifers. Interestingly, the composition of individuals in the HB and LB groups was similar at the two time points. This data not only demonstrates a significant variation in fly burdens between individuals, but also suggests that the fly burden in specific individuals is relatively constant over time. To explore the basis of an individual determinant for fly burden susceptibility, microbiome samples from each heifer in the HB and LB groups were collected from 3 sites (Rumen, Skin, Pharynx). These microbiomes will be measured using PCR to amplify and enrich the V3-V4 region of the 16S ribosomal RNA gene. Samples will be sequenced using the current generation MiSeq (Illumina) sequencing technology. Total microbial richness will be assessed through rarefaction analyses and diversity determined by Shannon's diversity indices. Analyses of relationships of fly loads to microbial composition will be examined using multivariate statistical approaches.

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Bartonellosis: One Health Perspectives on an Emerging Infectious Disease.

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Bartonellosis is a zoonotic infectious disease of worldwide distribution, caused by an expanding number of recently discovered *Bartonella* species. *Bartonella* spp. are transmitted by several arthropod vectors, including fleas, lice, sand flies, ticks, keds and biting flies. Prior to 1990, there was only one named *Bartonella* species (*B. bacilliformis*), whereas there are now at over 30 species, of which 17 have been associated with an expanding spectrum of animal and human diseases. Endocarditis, granulomatous inflammatory lesions, vasoproliferative vascular diseases and persistent blood borne infections are caused by bartonellosis in animals and human patients. Advances in diagnostic techniques have facilitated documentation of chronic bloodstream infections with *Bartonella* species in healthy and sick animals, and in immunocompetent and immunocompromised human patients. Importantly, bartonellosis appears to represent an occupational risk for veterinarians and other animal workers. The field of *Bartonella* research remains in its infancy and is rich in questions, for which patient-relevant answers are badly needed. It is possible that directed *Bartonella* research could substantially reduce animal and human suffering, which is seemingly associated with chronic debilitating disease processes. This lecture will emphasize the medical importance of *Bartonella* species as a cause of disease in animals and human patients and the benefits of using a One Health approach to this emerging infectious disease.

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Rickettsia felis in China

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Rickettsia felis is a recently described flea-borne spotted fever group Rickettsia that is an emerging human pathogen. Although there is information on the organism from around the world, there is no information on the organism in China.

We used a commercial ELISA to detect antibodies reactive against R. felis in blood samples and developed a PCR to detect the gltA of the organism in blood samples and external parasites.

We found reactive antibodies in people (16%; 28/180), dogs (47%; 128/271) and cats (21%; 19/90) and positive PCRs with DNA from people (0.1%; 1/822), dogs (0.8%; 8/1,059), mice (10%; 1/10), ticks (10%; 15/146), lice (16%; 6/37), fleas (95%; 57/60) and mosquitoes (6%; 25/428), but not from cats (0/135) or canine fecal swabs (0/43).

This is the first report of R. felis in China where there is serological and/or PCR evidence of the organism in previously reported (people, dogs, cats, ticks, fleas and mosquitoes) and novel species (mice and lice).

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A unique mechanism of protein-directed trans-activation of ribosomal frameshifting in arteriviruses

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Among the repertoire of mechanisms that viruses use to control or regulate their gene expression, non-canonical translation plays an important role, in particular for positive-strand RNA viruses whose genomic RNA serves a dual function as mRNA and genome. Recently, we uncovered that porcine reproductive and respiratory syndrome virus (PRRSV), and apparently most other arteriviruses, use an unusual -2 frameshifting (-2 PRF) signal directing efficient expression of a transframe protein (nsp2TF) from an alternative reading frame overlapping the viral replicase gene. The signal is also capable of directing -1 PRF, resulting in a truncated version of nsp2 (nsp2N) due to a stop codon adjacent to the shift site in the

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-1/+2 frame. The -2 and -1 PRF were found to occur with surprisingly high efficiencies (20% and 7%). Unusually, this arterivirus PRF signal lacks an obvious stimulatory RNA secondary structure. The minimal RNA sequence required for PRF was mapped within a 34-nucleotide region that includes the slippery sequence (G<sub>4</sub>GUU<sub>3</sub>UUU) and a downstream conserved CCCANCUCC motif. Remarkably, both frameshifts depend on the expression of a viral protein, specifically nsp1 $\beta$ , a PRRSV replicase subunit. Interaction of nsp1 $\beta$  with the PRF signal was demonstrated. Embedded in nsp1 $\beta$ 's papain-like autoprotease domain, we identified a highly conserved, putative RNA-binding motif that is critical for PRF trans-activation. These studies demonstrate for the first time that a protein can function as a trans-activator of ribosomal frameshifting. This could be a more widely employed mechanism, which might include viral strategies to regulate viral gene expression and/or modulate host cell translation upon infection.

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Regulatory role of the SAP-like motif of PRRSV nsp1 $\beta$  protein for innate immune response  
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Non-structural protein (nsp) 1 of porcine reproductive and respiratory syndrome virus (PRRSV) is a viral antagonist modulating the host innate immune response by blocking the expression of type I interferons (IFN- $\alpha$ / $\beta$ ) and inflammatory cytokines including tumor necrosis factor- $\alpha$ . Bioinformatics analysis identified a SAP (for SAF-A/B, Acinus, and PIAS) domain of 126-LxxxLxxxGL-135 in the nsp1 $\beta$  subunit, a protein structure associated with DNA-binding and nuclear retention of molecules involved in transcriptional control. Sequence alignments of nsp1 of PRRSV, equine arteritis virus (EAV), lactate dehydrogenase-elevating virus (LDV), and simian hemorrhagic fever virus (SHFV) indicated that the SAP domain was highly conserved in arteriviruses. The residues at L126, L130, G134, and L135 together with K124, R128, and R129 in the SAP domain were mutated to alanine to determine their role for IFN modulation. Luciferase reporter assays indicated that the IFN suppression by nsp1 $\beta$  was reverted when SAP mutants of L126A, R129, L130A, and L135A were expressed in cells, and either IFN regulatory factor (IRF) 3-dependent or NF- $\kappa$ B-dependent transcriptions were released from inhibition. G134A mutation did not alter the IFN suppression of nsp1 $\beta$ . These SAP mutants also exhibited altered subcellular localization from the nucleus to the cytoplasm in gene-transfected cells with the exception of G134A. The mutations contributing to impaired IFN regulatory activities were individually introduced into the PRRSV infectious clone, and infectious mutant viruses were generated for SAP mutants L126A and L135A. The nsp1 $\beta$  of mutant viruses, L126A and L135, were not detected in the nucleus which was consistent with the results in gene-transfected cells. In comparison with the wild-type virus, the SAP mutant viruses showed an impaired growth in cells, suggesting that the SAP domain contributes to PRRSV replication and plays an important role for viral immune evasion.

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The PRRSV-mediated inhibition of IFN $\alpha$  production by pig alveolar macrophages occurs at the post-transcriptional level via the activation of eIF-2 $\alpha$

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Using the porcine pulmonary alveolar macrophage (PAM) line ZMAC as well as primary PAMs, we examined the ability of North American (type II) PRRSV to elicit a type I interferon response and to suppress macrophage activation by the synthetic analog of dsRNA, poly(I:C). The ZMAC cell line is a non-transformed PAM permissive to PRRSV. The infection of ZMAC cells with PRRSV resulted in the phosphorylation of NF $\kappa$ B but not IRF3, which was congruent with the measurable secretion of TNF $\alpha$  but not of IFN $\alpha$ . Interestingly, while the stimulation of PRRSV-infected ZMAC cells with LPS yielded an additive TNF $\alpha$  response, stimulation with poly(I:C) under the same conditions resulted in a >50% decrease in the secretion of IFN $\alpha$ . Notably, the infection of ZMAC cells with PRRSV did not affect the ability of poly(I:C) to stimulate the transcription of IFN $\beta$ , IRF7 or IFN $\alpha$ , nor the phosphorylation of IRF3 or STAT1. Since there was no evidence that PRRSV inhibited the production IFN $\alpha$  at the transcriptional level, the possibility that the inhibition occurs at the translational level was explored. Infection of ZMAC or primary PAMs with PRRSV resulted in the phosphorylation of eIF-2 $\alpha$  by 8 to 10 hours post-infection. Time course studies revealed that the degree of IFN $\alpha$  inhibition correlates ( $r=0.97$ ) with the level of eIF-2 $\alpha$  phosphorylation. Since the activation of eIF-2 $\alpha$  should result in a generalized inhibition of cytokine synthesis, we set to ascertain the reason why an inhibitory effect on TNF $\alpha$  response to LPS was not observed. Analyses revealed that the TNF $\alpha$  response to LPS occurs with faster kinetics than the IFN $\alpha$  response to poly(I:C), with >60% of the total amount of cytokine secreted occurring by 4h in the case of TNF $\alpha$  as compared to 7h for IFN $\alpha$ . Since the PRRSV-induced activation of eIF-2 $\alpha$  occurs in earnest by 8 hours after infection, we found that the TNF $\alpha$  response to LPS could be inhibited >60% by PRRSV provided that enough time after infection had occurred (6h) before the activation with LPS. Our results indicate that PRRSV inhibits the production of IFN $\alpha$  in PAMs at the post-transcriptional level and is most likely mediated via the activation of eIF-2 $\alpha$ .

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Development of a synthetic porcine reproductive and respiratory syndrome virus strain that confers broader cross-protection

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Substantial genetic variation among PRRSV strains represents a major obstacle for the development of a broadly protective vaccine. We describe here a novel approach to generate a PRRSV vaccine strain that could confer broader cross-protection against divergent PRRSV isolates. We had initially obtained a set of 60 non-redundant, full-genome sequences of type-II PRRSV. After that, we generated the consensus genome (designated as PRRSV-CON) by aligning the 60 PRRSV full-genome sequences, followed by selecting the most common nucleotide found at each position of the alignment. Our analysis demonstrates that the PRRSV-CON has the highest degree of sequence identity to the PRRSV field-isolates when compared to any current PRRSV vaccine strains, both at the full-genome level and the individual gene level. Next, we chemically synthesized the PRRSV-CON genome and assembled it into a bacterial plasmid under the control of the T7 promoter. The resulting PRRSV-CON cDNA clone is fully infectious. Viable virus is consistently produced after MARC-145 cells are transfected with the RNA transcript produced from the PRRSV-CON cDNA clone. Moreover, the PRRSV-CON virus replicates as efficiently as our prototype PRRSV strain FL12, both in vitro and in vivo. Importantly, primary infection of pigs with PRRSV-CON virus confers significantly broader protection than the prototype PRRSV strain FL12 when tested upon subsequent challenge with a third unrelated heterologous PRRSV strain. Collectively, our data

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demonstrate that the PRRSV-CON virus can serve as a potential vaccine candidate for the development of a novel PRRS vaccine with broader cross-protection.

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Characterization of entry events during bile acid-mediated porcine enteric calicivirus replication

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**Purpose:** Replication of porcine enteric calicivirus (PEC) in LLC-PK cells is unique because bile acids enable the growth of PEC. Since bile acids are present in high concentrations in the intestines, where PEC replicates, bile-acid dependent PEC replication in cell culture may reflect the host-pathogen interaction occurring in the animals. The aims of our study are to elucidate the mechanism of bile acid-dependent PEC replication, which may also yield an insight to the isolation of fastidious viruses that are related to PEC, such as human noroviruses.

**Methods:** Confocal microscopy, time of addition study and viral replication assay with various siRNA as well as small molecule inhibitors were used to elucidate bile acid-mediated PEC replication in cell culture.

**Results:** Our findings based on confocal microscopic study and time of addition assays show that bile acids are required for viral escape from the endosome-like compartments into cytoplasm prior to initiation of viral replication. Since it is known that bile acids activate acid sphingomyelinase (ASM) and induce ceramide formation, which destabilizes lipid bilayer and promotes membrane fusion, we further investigated the role of ASM in bile acid-mediated ceramide formation and PEC replication. We found that treatment of cells with bile acids led to formation of ceramide in the membranes of the endosome-like compartments in LLC-PK cells mediated by ASM activation. We also demonstrated that siRNA or small molecule inhibitors of ASM significantly reduced PEC replication by inhibiting viral escape from the endosome-like compartments in the cells. Inhibition of ASM activity also significantly reduced the replication of other caliciviruses including feline calicivirus and murine norovirus.

**Conclusions:** Our finding suggests novel host-pathogen interactions involved in ASM and viral escape from endosome-like compartments as well as a common mechanism utilized by caliciviruses during virus entry to cells.

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The spike protein furin cleavage site in feline coronavirus is not the sole determinant of conversion to feline infectious peritonitis virus

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Feline coronavirus (FCoV) infection is ubiquitous in domestic cat populations. As yet unknown mutations result in extra-intestinal replication of the virus, causing feline infectious peritonitis (FIP), a highly fatal immune-mediated disease. Recently, it was proposed that mutations within the furin cleavage site of FCoV spike protein that reduce the ability of the cellular proteinase, furin, to cleave the spike protein, convert FCoV to FIP. To evaluate this hypothesis, we developed a quantitative fluorescence resonance energy transfer (FRET)-PCR that amplifies a fragment of the spike gene sequence encoding the furin cleavage site. In this study, we tested samples from the cats that had clinical signs of FIP and were positive for coronavirus RNA in their peritoneal effusions by this PCR. Amplification products were sequenced, and the amino acid sequence of the furin cleavage site was determined. Out of 25 amplification products, 14 (56%) had critical mutations within the furin cleavage site that are known to reduce cleavage efficiency. The sequence of the canonical core motif of the FCoV furin cleavage site is R-R-S/A-R-R-S. In our study, these 14 samples variably showed mutations at each of the positions of the core motif. Thirteen out of 14 samples harbored only one mutation within the core motif, whereas one sample harbored two mutations. The 3<sup>rd</sup> and 5<sup>th</sup> position had the highest mutation frequency (21.4% each), with changes from arginine (R) to leucine (L), and from serine (S) to proline (P), respectively. These results indicate that the presence of a mutation within the furin cleavage site is not a requirement for conversion of FCoV to FIPV. To further assess the relevancy of mutations within the FCoV spike protein furin cleavage site to FIP infection, an FIP-unbiased population of FCoV amplification products will be collected from juvenile shelter cats, and mutation frequency will be compared to that of FIP viruses.

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Assessment of viremia and tissue distribution of porcine epidemic diarrhea virus in weaned pigs after experimental infection

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Porcine epidemic diarrhea virus (PEDV) has caused substantial pre-weaned pig mortality since it was first recognized in US in 2013. Although infected pigs typically do not have clinical diarrhea after 7 to 10 days post infection, pigs still shed the virus in feces for an extended period of time after that. The objective of this study was to assess the distribution of PEDV in various tissues of pigs following experimental inoculation with PEDV to determine if this could be a potential contributor to the extended viral shedding. Sixty-three, 3-week-old pigs were allocated into control (n=27) and challenged (n=36) groups. Challenged pigs were orogastrically inoculated with 1 ml of 1x10<sup>3</sup> PFU/ml of PEDV isolate (US/Iowa/18984/2013). Three control and four challenged pigs were necropsied on days post inoculation (dpi) periodically until 35 dpi. Various enteric and non-enteric tissues were collected. Sera were collected from necropsied pigs and from all remaining pigs starting at 5 dpi. All samples were tested by RT-qPCR and/or immunohistochemistry (IHC). Viremia was detected by PCR in some (50%) as early as 1 dpi and was no longer detectable after 7 dpi except one pig which was viremic at 14 dpi. PEDV RNA was detected in small intestine of all pigs at 1 dpi and was still detectable in 50% of the remaining pigs at 28 dpi. PEDV RNA was also detected in other tissues (stomach, colon, lung, heart, spleen, kidney, mesenteric lymph node) as early as 1 dpi but was no longer detectable after 14 dpi except mesenteric lymph node in which PEDV RNA continued to be detected by 28 dpi. PEDV antigen was also detected in mesenteric lymph node and colon by IHC but at a less frequency as compared to the PCR result, suggesting that IHC is less sensitive than PCR. Overall fecal shedding is attributed to viral replication in small intestine; however, a viremic phase appears to result in wide distribution of PEDV to non-enteric tissues. The immunobiological significance of detecting PEDV in the mesenteric lymph node for a prolonged time warrants further investigation as the tissue plays a role in local immunity.

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Sequencing analysis of recently outbreaked porcine epidemic diarrhea virus in vietnam

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Porcine epidemic diarrhea virus (PEDV) is a highly devastating enteric disease that is characterized by acute diarrhea, dehydration and significant mortality in swine. The PEDV, a member of the family *Coronaviridae*, is an enveloped, positive sense, single-stranded RNA virus. Recently clinical outbreaks of diarrhea were emerged in local farm of Hanoi, Vietnam. In this study, we carried out RT-PCR (targeting M gene) and quantified the viral titer using real-time PCR (qPCR) on feces and intestine sample from Vietnam. 16/36 samples were positive for PEDV and average TCID<sub>50</sub>/ml was confirmed. Spike (S) protein, a glycoprotein peplomer on the viral surface, is an important for processing of inducing neutralizing antibodies and specific receptor binding and several neutralizing peptides have been identified in the spike protein of PEDV. To understand and analysis of genetic relationship with other strains PEDV in Vietnam, Sequencing and phylogenetic analysis was conducted on their S gene. Interestingly, comparative analysis of recent vietnam PEDV revealed that mutation within the S gene existed and in comparison with other strains revealed that the sequence of S protein of vietnam strain exhibited high homology both at the nucleotide level and at the deduced amino acid level. These results show that epidemiological differences are originated in difference of sequence and more study is required to reveal the viral pathogenicity by difference of sequence.

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Pathogenesis of US porcine deltacoronavirus strains FD22 and FD100 in gnotobiotic pigs.

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To understand the disease progression of newly emerged porcine deltacoronavirus (PDCoV), we studied pathogenicity of two US PDCoV strains (FD22 and FD100) confirmed free of other viral pathogens and bacteriologically sterile in gnotobiotic (Gn) pigs. Six 11- to 14-day-old pigs were randomly assigned to two groups: PDCoV-infected (n=3 for FD22; n=2 for FD100) and negative control (n=1). Gn pigs were inoculated orally with 8.8-11.0 log<sub>10</sub> genomic equivalents (GE) of FD22 or FD100. Clinical signs and virus shedding were monitored after inoculation until necropsy. All infected pigs exhibited acute severe diarrhea and vomiting between post-inoculation hour (PIH) 21-24, followed by dehydration. A FD22-infected pig, followed longer-term, showed moderate to severe diarrhea up to PIH 144. The pig had prolonged fecal viral RNA shedding (peak titer of 8.5 log<sub>10</sub> GE/ml at PIH 48) at least until 23 days after inoculation. At PIH 72-120, pathologic lesions were limited to the small and large intestines, and identified microscopically as moderate to severe atrophic enteritis. Mean jejunal ratios of villous height to crypt depth (VH:CD) in infected pigs ranged from 1.4 to 3.6. PDCoV antigens were observed in villous epithelial cells of the small (duodenum to ileum) and occasionally, large intestines by immunofluorescent (IF) staining using a hyperimmune Gn pig antiserum against FD22. By in situ TUNEL assay, PDCoV appeared to not induce apoptotic death of enterocytes in the small intestine of infected pigs. Our data suggest that the US strains of PDCoV acutely infects the entire intestine, although principally the jejunum and ileum, leading to severe atrophic enteritis. To present, differential diagnosis of porcine epidemic diarrhea virus and PDCoV is critical to control virus epidemic diarrhea in US swine farms.

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Development and validation of an indirect porcine deltacoronavirus (PDCoV) anti-IgG ELISA based on the S1 portion of the spike protein and confirmation that PDCoV infection in U.S. pigs is low and has been present since 2010

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Porcine deltacoronavirus (PDCoV) was first diagnosed in the United States in early 2014 resulting in an urgent need for reliable, fast, and cost-effective tools to perform diagnostic investigations. An indirect PDCoV anti-IgG enzyme-linked immunosorbent assay (ELISA) based on the S1 portion of the spike protein was developed and validated on paired serum samples from commercial pigs with confirmed PDCoV infection. A distinct seroconversion to PDCoV was detected in naturally infected pigs. Receiver operating characteristic analysis estimated the cut off as 0.385 with a diagnostic sensitivity of 90% and specificity of 90% for IgG antibodies on serum samples. A total of 355 samples from 2014 were tested including samples with confirmed infection against other porcine coronaviruses such as porcine epidemic diarrhea virus, transmissible gastroenteritis virus or porcine respiratory coronavirus. Among the 355 serum samples which originated from 51 farms, PDCoV antibodies were detected in 7.9% of the samples. On positive farms, 20 to 60% of the samples were PDCoV positive. In addition, archived serum samples were also tested and the results indicate that PDCoV circulated in U.S. pigs at least since 2010.

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African swine fever virus (ASFV) rp30 ELISA detects antibody in serum and/or oral fluid specimens

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ASFV is of concern because of its high mortality rate, its severe economic impact, and its recent rapid geographic expansion. Prevention and control of ASFV is complicated by the absence of effective vaccines. Serology is useful for ASFV diagnosis and surveillance because antibodies are a definitive indication of infection and are detectable for a prolonged period of time. An earlier study showed that ASFV antibodies could be detected in oral fluids and suggested that oral fluids could serve as a suitable specimen for ASFV surveillance.

The objective of this study was to develop a dual-matrix serum/oral fluid ASFV p30 antibody ELISA kit. The recombinant p30 antigen used in the ELISA was selected by evaluating the serum antibody response of ASFV-infected pigs against 3 His-tagged fusion recombinant polypeptides (rp30, rp54, rp72) using a multiplex fluorescent microbead-based immunoassay (FMIA; Luminex® Corporation).

Serum and oral fluid antibody-positive samples were generated by experimental inoculation of 9 pigs with an attenuated ASFV isolate (NHV) that produces chronic infection. Paired oral fluid and serum samples were sequentially collected from individual pigs (DPI 0, 6, 12, 15, 19, 26,

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33, 40, 47, 54, and 61). ELISA specificity was evaluated using 200 serum and oral fluid samples submitted to the Iowa State University Veterinary Diagnostic Laboratory from swine herds in the U.S., i.e., a known ASFV-negative population.

IgG antibodies were detected by DPI 12 in both serum and oral fluid specimens. Evaluation of ASFV negative field samples showed specificities of 99.5% and 100% for serum and oral fluid samples, respectively. Thus, the test detects ASFV antibodies in either oral fluid or serum samples, yet is highly specific for both specimen types. Given the increased surveillance efficiency provided by oral fluid sampling and the ability to corroborate results using serum samples, the ASFV rp30 antibody would be a highly useful under conditions that warrant ASFV surveillance.

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Acute infection with bovine viral diarrhea virus causes depletion of WC1+  $\gamma\delta$  T cells in lymphoid tissues in beef calves

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Bovine viral diarrhea virus (BVDV) acute infection has been associated with lymphoid depletion, leukopenia, and impairment of leukocyte function.  $\gamma\delta$  T cells represent an important T lymphocyte population in cattle. Previous studies showed an increased number of circulating  $\gamma\delta$  T lymphocytes after BVDV infection or vaccination. The objective of this study was to determine the abundance of  $\gamma\delta$  T lymphocytes in lymphoid tissues during acute infection with high or low virulence non-cytopathic BVDV in beef calves. Twenty-four beef calves were randomly assigned to 1 of 3 groups: LV (n=8): animals challenged intranasally (IN) with low virulence BVDV-1a (strain SD-1). HV (n=8): animals challenged IN with high virulence BVDV-2 (strain 1373), and control (n= 8): animals inoculated with cell culture medium. On day 5 post-challenge, animals were euthanized and samples from spleen and mesenteric lymph nodes (MLN) were collected to assess the abundance (counting ten 40X fields) of  $\gamma\delta$  T cells as detected by immunohistochemistry using an anti-bovine WC1 monoclonal antibody. A higher proportion of calves challenged with BVDV (LV and HV groups) showed signs of apoptosis and cytophagy in MLN and spleen samples compared to the control group. A significantly lower number  $\gamma\delta$  T cells was observed in calves in HV (spleen:  $27.6 \pm 8.4$  cells; MLN:  $50.8 \pm 24.1$  cells) and LV groups (spleen:  $54.1 \pm 14.6$  cells; MLN:  $36.5 \pm 21.9$  cells) compared to the control calves (spleen:  $225.5 \pm 48.4$  cells; MLN:  $227.4 \pm 42.2$  cells;  $P < 0.05$ ). In conclusion, acute infection with high or low virulence BVDV caused depletion of WC1+  $\gamma\delta$  T cells in lymphoid tissues in beef calves.

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The bovine immunodeficiency virus Rev protein: characterization of the multimerization domain using the bimolecular fluorescence complementation (BiFC) technology

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Bovine immunodeficiency virus (BIV) is a lentivirus of the Retroviridae family which shares morphologic, genetic, antigenic and/or biologic properties with human immunodeficiency virus type 1 (HIV-1) and other animal lentiviruses. The BIV genome contains open reading frames that may encode nonstructural/regulatory proteins. Among the latter proteins is Rev. The HIV-1 Rev protein shuttles between the nucleus and the cytoplasm to regulate the virus replication. Rev directly binds to unspliced and partially spliced viral RNA via the cis-acting Rev Response Element (RRE) sequence. Subsequently, Rev oligomerizes cooperatively and interacts with the cellular nuclear export receptor CRM1. Here we report the characterization of the multimerization domain of this protein. Protein cross-linking experiments using recombinant Rev showed that BIV Rev multimerizes in vitro. By using a series of Rev deletion mutants, two regions encompassing amino acids (aa) 1 to 30, and 90 to 110 of the BIV Rev sequence appeared to be necessary for the multimerization of the protein in vitro. The use of a mutant form of BIV Rev that does not localize to the nucleus together with wild-type Rev indirectly showed that the protein also multimerizes in living cells as seen by confocal microscopy analysis. These results were confirmed by using a bimolecular fluorescence complementation (BiFC) assay. Experiments are under way to identify more precisely the residues that mediate the multimerization of BIV Rev by using the BiFC technology.

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Efficacy of M2e-based vaccine in murine, avian and swine models

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The M2e protein of influenza virus is highly conserved among strains and has been an attractive vaccine target for broad protection against different subtypes. Although M2e-based vaccine has been explored for more than two decades, the mouse model has been used almost exclusively and limited studies have been conducted in swine and chickens, the two most important species in influenza epidemiology. In this study, we utilized flexible norovirus P particle as a novel influenza vaccine platform to express M2e (M2e-PP). We tested immunogenicity and protective efficacy of M2e-PP in mouse, pig, and chicken model using different route of vaccination. In all 3 species, a single intramuscular or subcutaneous vaccination induced a detectable anti-M2e antibody response which increased significantly following booster vaccination. In contrast to mice, intranasal vaccination of M2e-PP in chicken and pig did not induce detectable IgG antibody. In mouse, both intranasal and subcutaneous vaccinations prevented clinical signs (body weight loss) and mortality, although intranasal route of vaccination outperformed subcutaneous vaccination in terms of level of protective efficacy. In chickens and swine, M2e-PP shows 1-2 log reduction in virus shedding, and intramuscular or subcutaneous vaccination using commercial oil adjuvant outperformed intranasal vaccination. We also observed that the addition of M2e protein to inactivated vaccine conferred improved protection compared to single regime vaccination suggesting a possible approach to modify traditional vaccination strategy. Also in chickens, addition of M2e vaccine enhanced the hemagglutination inhibition (HI) antibody response induced by inactivated vaccine. Our study shows clear difference in efficacy of M2e-based vaccine depending on the animal species and vaccination route.

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Potential of endogenously produced and exogenously administered interferon as adjuvant for influenza vaccine in animals

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Type I interferon is primarily known for its antiviral activity. It also serves as a signal linking innate and adaptive immunity by enhancing the development of adaptive immune responses. Our studies in chickens, mice and pigs demonstrated that influenza virus mutants that encode C-terminally truncated NS1 proteins (*NS1-truncation* mutants) are highly attenuated, immunogenic and thus excellent live-attenuated influenza vaccine (LAIV) candidates. When the effectiveness of selected LAIV candidates were evaluated in animals in terms of HI antibody titers, clinical signs and replication and shedding of heterologous challenge viruses, it was directly related with the amount of interferon induced in cell culture or the vaccinated host but not the size of the truncated NS1. These data suggest that endogenously-induced IFN is required for the self-adjuvant activity of effective *NS1-truncation*-based LAIV candidates. To test the adjuvant effects of exogenously administered type I interferon, we vaccinated 4 weeks-old chickens subcutaneously with inactivated influenza vaccine and provided them with interferon in drinking water at a dose of  $1 \times 10^5$  Units per bird per day for 14 days. Nine of ten interferon-treated birds had seroconverted by 8 day post treatment while only 2 out of 10 birds provided with plain drinking water had seroconverted. However, the number of seroconverted birds and HI antibody titers at 14 days post treatment were not significantly different between the treated and untreated groups. Our data indicate that exogenously administered type I interferon has a priming effect with potential to provide an early and uniform protection of birds against influenza virus challenge.

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Poly I:C adjuvanted inactivated swine influenza vaccine induces heterologous protective immunity in pigs

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Swine influenza is widely prevalent in swine herds in US and Europe causing enormous economic losses and public health threat. Pigs can be infected by both avian and mammalian influenza viruses thus are source of generation of reassortant influenza viruses capable of causing pandemics in humans. Current commercial vaccines provide satisfactory immunity against homologous viruses; however, protection against heterologous viruses is not adequate. In this study, we evaluated the protective efficacy of intranasal Poly I:C adjuvanted UV inactivated bivalent swine influenza vaccine consisting of Swine/OH/24366/07 H1N1 and Swine/CO/99 H3N2, referred as PAV, in maternal antibody positive pigs against antigenic variant and heterologous swine influenza virus challenge. Groups of three-week-old commercial pigs were immunized intranasally with PAV or commercial vaccine (CV) twice at 2 weeks intervals. Three weeks after the second immunization, pigs were challenged with antigenic variant Swine/MN/08 H1N1 (MN08) and heterologous Swine/NC/01 H1N2 (NC10) swine influenza virus. Antibodies in serum and respiratory tract, lung lesions, virus shedding in nasal secretions and virus load in lungs were examined. Intranasal administration of PAV induced challenge viruses specific HI and IgG antibodies in the serum and IgA and IgG antibodies in the respiratory tract. Importantly, intranasal administration of PAV provided protection against antigenic variant MN08 and heterologous NC10 swine influenza viruses as evidenced by significant reduction in lung virus load, gross lung lesions and significant reduced shedding of challenge viruses in nasal secretions. Thus, these results suggest that Poly I:C or its homologues may be effective as vaccine adjuvants capable of generating the cross-protective immunity against antigenic variants/heterologous swine influenza viruses in pigs.

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Viral Pathogenesis: Lessons Learned from Newcastle Disease Virus.

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Newcastle Disease Virus (NDV) causes a highly contagious and devastating disease in poultry and other birds that has a worldwide distribution. NDV strains vary greatly in virulence. The presence of multi basic residues at the proteolytic cleavage site of the fusion (F) protein has been shown to be a primary determinant differentiating virulent versus avirulent strains. However, there is a wide variation on the virulence among virulent NDV strains. These observations suggest that additional viral factors contribute to virulence. Using reverse genetic techniques, we have exchanged individual genes between NDV and avirulent avian paramyxovirus serotype-2 (APMV-2) and between velogenic (highly virulent) and mesogenic (moderately virulent) NDV strains. The results from MDV and APMV-2 gene swapped viruses showed that the F and HN proteins together determine the replication, tropism, and pathogenicity of avian paramyxoviruses. The results from velogenic and mesogenic gene swapped viruses showed that the F protein was the major individual contributor and was sometimes augmented by the homologous M and HN proteins. The dramatic effect of F as individual contributor was sometimes augmented by the homologous N and P proteins. Leader and trailer regions did not appear to contribute to the virulence between velogenic and mesogenic strains.

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Rescue and characterization of a new re-assortant influenza virus strain H5N1

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Purpose. Circulation of the H5N1 subtype of highly pathogenic avian influenza viruses (HPAIVs) around the world has caused economic losses to the poultry. Because the H5 subtype has been continuously evolving, the antigenicity of the stockpiled vaccine might not adequately match HPAIVs that will appear in the future. To achieve adequate efficacy of the inactivated vaccine, antigenic matching between a vaccine candidate strain and a circulating virus is required. Methods. In this study, reverse genetics was applied to engineer a reassortant vaccine candidate strain against HPAIVs of the H5 subtype. The new strain recPR8-H5N1 contained the HA gene from the Russian HPAIV A/Kurgan/05/2005 (H5N1), the NA and internal genes from A/Puerto Rico/8/34 (H1N1). Results. The strain recPR8-H5N1 demonstrated antigenic specificity (H5), high proliferation rate in 10 day chicken embryos, and was lethal for the embryos in 36 hours. An inactivated emulsified vaccine based on the strain recPR8-H5N1 elicited high antibody titers and protected 6 week old chickens from lethal challenge with the HPAIV A/Kurgan/05/2005 (H5N1).



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on day 21 after single immunization. Infection of non-vaccinated birds with strain recPR8-H5N1 did not cause any pathology, and the virus was not detected by PCR in blood and cloacal swabs on day 7 p.i. Specific weak seroconversion caused by infection with the strain recPR8-H5N1 was registered on day 14 p.i. Conclusions. A new influenza virus strain was obtained with altered properties. The new strain does not belong to HPAIV, has high reproductive potential and antigenic identity to the HPAIV subtype H5.

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Naturally truncated NS gene of H3N8 equine influenza virus attenuates the virulence of the A/Puerto Rico/8/34 virus

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Equine influenza virus (EIV) causes a highly contagious disease of horses and other equid, and also transmits into dogs. Recently, we isolated NS gene-truncated H3N8 EIV from vaccinated horse showing symptoms of respiratory disease in South Korea, and the EIV showed low viral growth kinetics. In order to elucidate influences of NS gene truncation on reduced viral virulence, reverse genetics were applied to generate different NS recombinant virus utilizing an identical [A/Puerto Rico/8/1934(H1N1), PR8] virus. We have analyzed and cytokine production in mice, and found that naturally truncated NS gene leded incompetent and cytokine production compared to other rescued PR8 containing intact NS genes. This study demonstrated that the partially deleted NS gene is responsible for the low pathogenesis and inefficient viral replication of the Korean H3N8 EIV.

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Role of Ebola virus matrix protein in regulating cellular innate immune response

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Innate immune response acts as the first line of defense and involves type-I interferon, nitric oxide, autophagy, and apoptosis. Ebola virus (EBOV), a negative sense ssRNA virus (NSV) of family Filoviridae, causes severe hemorrhagic fever in humans, with case mortality rates up to 90%. Current outbreak of Ebola virus disease (EVD) resulted in 1350 deaths until August 19, 2014 in West Africa and still continuing. In the absence of well-characterized therapies for EVD, the search for effective therapeutic against EBOV is imperative. The matrix protein (VP40), a structural protein of EBOV is essential for virus replication and budding, and is known to interact with various host proteins such as ubiquitin ligase and cytoskeletal proteins. Viral matrix proteins of many NSV are known to shuttle between the nucleus and cytoplasm, and also antagonize innate immune response. EBOV VP40 lacks classical nuclear localization signal (NLS), but has nuclear export signal (NES) for nuclear-cytoplasmic trafficking. Despite this, it has been shown to localize to the nucleus but the biological significance is unknown. The mechanism by which EBOV VP40 translocates to the nucleus, role of nuclear localization in virus budding or its influence on the induction of host transcription factors remains undefined. By confocal microscopy, we found that VP40 transiently localized to the nucleus at 6-10 h post-transfection with subsequent localization to the cytoplasm and then to the plasma membrane by 16-24 h. The effects of VP40 in regulating various transcription factors were also examined and these will be discussed in relation to the induction of innate immune response against EBOV, virus life cycle and developing targeted therapeutics.

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