PROGRAM and PROCEEDINGS

of the 93rd Annual Meeting
December 2, 3 and 4, 2012

Marriott, Downtown Magnificent Mile
Chicago, Illinois

Robert P. Ellis, Executive Editor

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

The 93rd Annual Meeting of the CRWAD is dedicated to

Dr. William C. Wagner

Proceedings Distributed by CRWAD

CRWAD 93rd ANNUAL MEETING-2012

December 2 – 4, 2012

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

Registration - 5th 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 2, 6-8 PM – Grand Ballroom Salon III - 7th Floor
Introduction of CRWAD Officers and Dedicatee, Poster Session I

Student Reception – Students and invited guests - 5:00 PM – 5:45PM, Salon II Room, 7th Floor

Business Meeting - Chicago Ballroom A/B/C/D 5th Floor

11:45 AM - 12:30 PM Tuesday, December 4
Dedication of the 2012 meeting to Dr. William Charles (Bill) Wagner
Introduction of New Members and Graduate Student Awards Presentations

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

Speaker Ready Room is: Streeterville Room (2nd floor) - Sunday, Dec. 2 - Monday, Dec. 3

Marriott Hotel Section	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 - 010	Avenue Ballroom 011 – 022	
Biosafety and Biosecurity		Denver/Houston 023 – 030	
Companion Animal Epidemiology			Denver/Houston 033 – 040
Epidemiology and Animal Health Economics	Salons A/B/C/D 041 – 052	Salons A/B/C/D 053 – 063	Salons A/B/C/D 031, 032, 064 – 071
Food and Environmental Safety	Salon E 072 – 083	Salon E 084 – 094	Salon E 095 – 102
Gastroenteric Diseases	Michigan/Michigan State 103 – 109	Michigan/Michigan State 110 – 114	
Immunology	Salons F/G/H 115 – 123	Salons F/G/H 124 – 131	Salons F/G/H 132 - 135
Respiratory Diseases	Indiana/Iowa 137 – 148	Indiana/Iowa 149 – 156	Indiana/Iowa 157 – 164
Vector-Borne and Parasitic Diseases	Denver/Houston 165 – 174		
Viral Pathogenesis	Los Angeles/Miami 175 – 186	Los Angeles/Miami 187 – 195	Los Angeles/Miami 196 – 202
Posters* in Grand Ballroom	Salon III-7th Floor Sun. 6:30 - 8 PM	Salon III-7th Floor Mon. 5 - 6:30 PM	

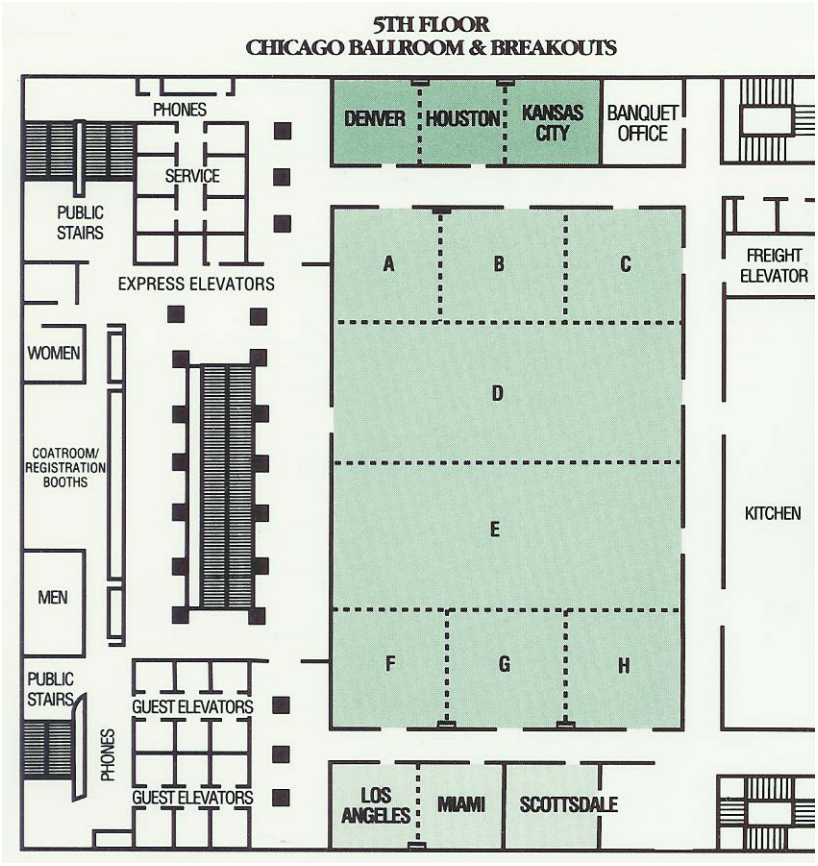
***SUNDAY POSTER PRESENTERS**: Poster boards will be available for poster assembly by 4 PM Sunday. Posters for the Bacterial Pathogenesis, Biosafety and Biosecurity, Companion Animal Epidemiology, Epidemiology and Animal Health Economics, and Gastroenteric Diseases Sections will be presented Sunday from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

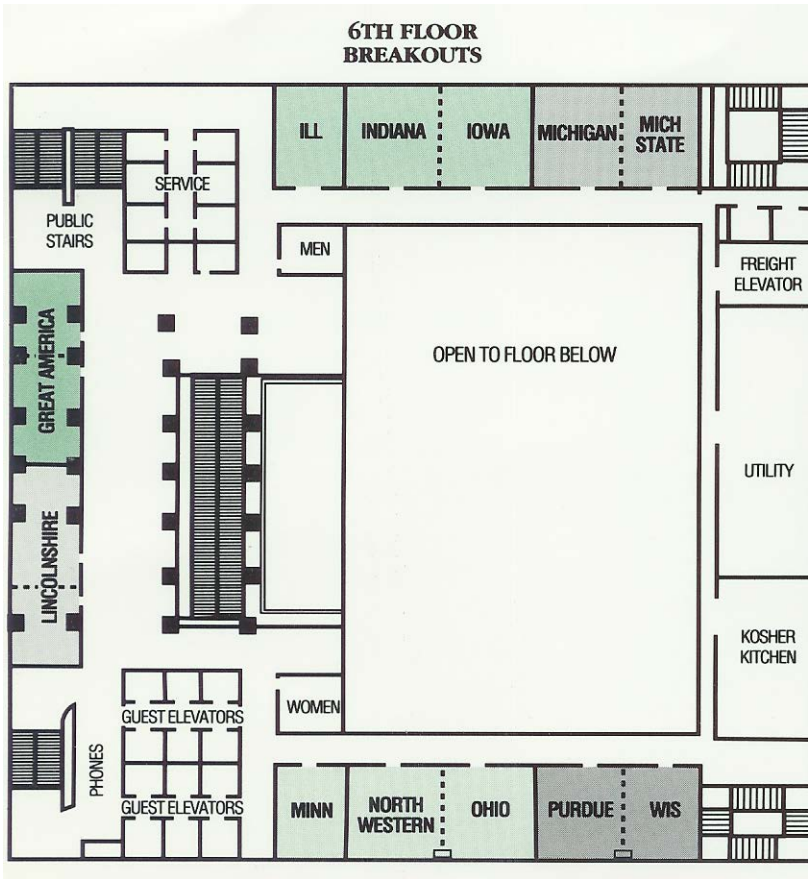
***MONDAY POSTER PRESENTERS**: Poster boards will be available for poster assembly by noon Monday. Posters for the Food and Environmental Safety, Immunology, Respiratory, Vector-Borne and Parasitic Diseases, and Virology Sections will be presented Monday from 5:00-6:30 PM. Please remove your posters 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.

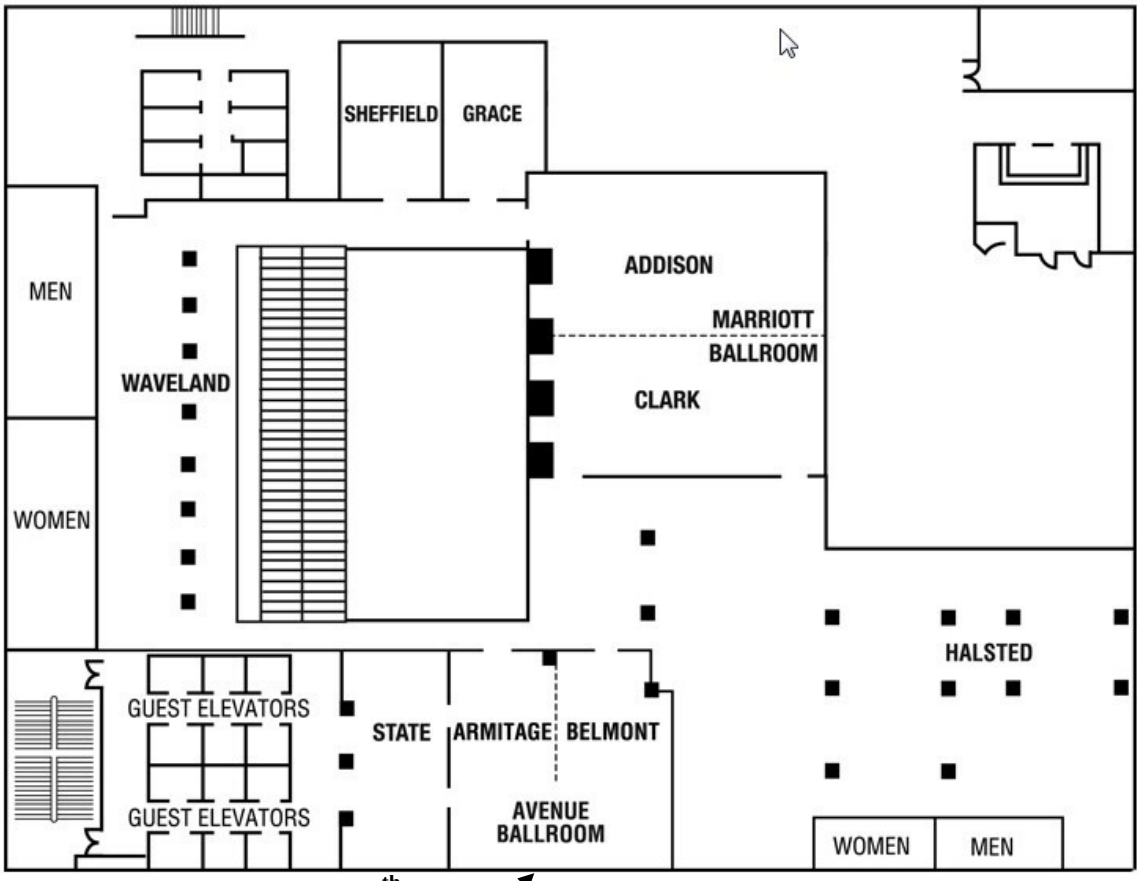
Poster Boards are 4 ft tall x 8 ft wide. Poster presenters must furnish their own tacks.

Chicago Marriott, Floor Plan - 5th and 6th Floors

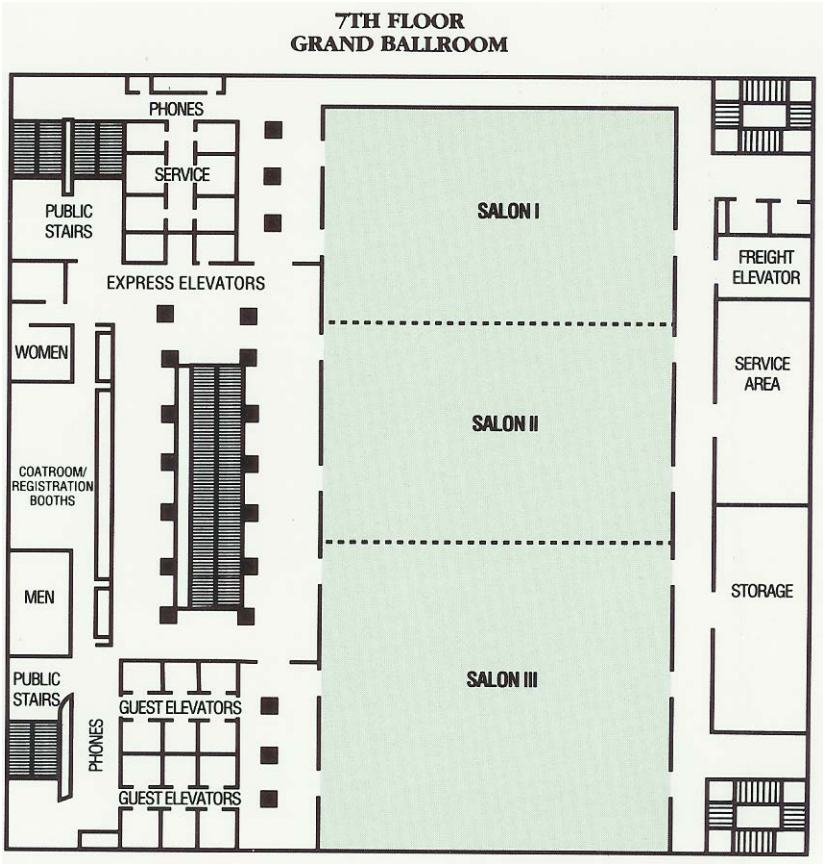




Chicago Marriott Floor Plan – 4th and 7th Floors



Avenue Ballroom on 4th Floor



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

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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 ten Sections, according to the primary topic of the presentation: Bacterial Pathogenesis, Biosafety and Biosecurity, Companion Animal Epidemiology, Epidemiology and Animal Health Economics, Food and Environmental Safety, Gastroenteric Diseases, Immunology, 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 500 to 550 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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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 2012 Dedicatee are listed below:

W. R. Hinshaw	1974	S. H. McNutt	1975
H. C. H. Kernkamp	1976	R. W. Dougherty	1977
C. H. Brandley	1978	S. F. Scheidy	1979
A. G. Karlson	1980	I. A. Merchant	1981
L. C. Ferguson	1982	Fred Maurer	1983
Carl Olson, Jr.	1984	Charles Cunningham	1985
Ben S. Pomeroy	1986	Norman Levine	1987
Earl Splitter	1988	Marvin J. Twiehaus	1989
R. Allen Packer	1990	Donald A. Barnum	1991
Alvin F. Weber	1992	E. O. Haelterman	1993
Erwin M. Kohler	1994	Edward H. Bohl	1995
Lyle E. Hanson	1996	Gordon R. Carter	1997
J. Brian Derbyshire	1998	Bernard C. Easterday	1999
Leroy Coggins	2000	David P. Anderson	2001
Johannes Storz	2002	Alexander J. Winter	2003
Harley W. Moon	2004	William L. Mengeling	2005
Leland E. Carmichael	2006	Richard F. Ross	2007
Sidney A. Ewing	2008	Norman F. Cheville	2009
Samuel K. Maheswaran	2010	Donald G. Simmons	2011
William C. Wagner	2012		

2012 CRWAD Dedicatee – William C. (Bill) Wagner

William C. Wagner, DVM, PhD, Dipl. ACT

Dr. Wagner received the DVM degree in 1956 and the PhD degree in 1968, both from Cornell. He is a member of several honor societies: Alpha Zeta, Phi Zeta, Phi Kappa Phi, Gamma Sigma Delta and Sigma Xi. He is a Charter Diplomate of the American College of Theriogenologists and an Honor Role member of the American Veterinary Medical Association. He is a Distinguished Scholar of the National Academy of Practice-Veterinary Medicine. He is a member of several scientific societies including the Society for the Study of Reproduction (Charter Member), Society for the Study of Fertility, American Society of Animal Science, American Physiological Society, Conference of Research Workers in Animal Disease (President, 1988-89), International Congress on Animal Reproduction (President, 1988-96) and the American Association of Veterinary Laboratory Diagnosticians.

He was the recipient of an NIH Postdoctoral Fellowship at Cornell University in 1965-68, a Senior U. S. Scientist Awardee of the Alexander von Humboldt Foundation in 1973-74, a Senior Fulbright Research Professorship in Germany (1984-85), and received the David Bartlett Award of the American College of Theriogenologists in 1995 and the William P. Switzer Award from Iowa State University for Meritorious Service in Veterinary Medicine in 1999. Dr. Wagner has been listed in Who's Who in Frontiers of Science and Technology, American Men and Women of Science, Who's Who in Veterinary Medicine and Who's Who in America.

After one year in a general practice in Interlaken, NY with Dr. Howard K. Fuller, Dr. Wagner was a research associate in veterinary pathology with Dr. Kenneth McEntee, and then completed the PhD degree in physiology in 1968 with Dr. William Hansel at Cornell. He then joined the faculty of the Veterinary Medical Research Institute at Iowa State University in January 1968 as an Assistant Professor, rising to Full Professor in 1976. In 1977, he moved to the University of Illinois as Head, Dept. of Veterinary Biosciences and in 1990 became Associate Dean, Research and Graduate Studies. During this time period Dr. Wagner served as a program manager in competitive grants in animal reproduction at the USDA-CSREES and as a member of the Study Section on Fetal Development at the NIH. In 1990-93 he also was involved in the development of the competitive grants program in animal health at the USDA-CSREES agency. Dr. Wagner was named Leader of the Section on Animal Systems and National Program Leader for Veterinary Medicine at the USDA-CSREES in 1993, a position he held until retirement in 2002. Dr. Wagner then accepted a position as Visiting Professor at The Ohio State University, working on strategic planning and research funding as well as continuing with a major effort in further development of the National Animal Health Laboratory Network, which had been initiated with his leadership in 2002 while still at USDA. In August 2007, Dr. Wagner accepted the appointment as Dean, School of Veterinary Medicine, St. Matthew's University, Grand Cayman, Cayman Islands, BWI. He left this position in December 2011 and is now Dean Emeritus at the School.

2012 CRWAD Dedicatee – William C. (Bill) Wagner

Dr. Wagner has served as an international consultant for IICA in Brazil (1982) and The Winrock Foundation in Pakistan in 1990. In addition he has participated in scientific meetings and presented short courses on animal reproduction in Brazil on two occasions and given numerous scientific papers and lectures at international meetings and universities.

With respect to mentoring of trainees, he has served as a mentor for four postdoctoral fellows (3 of them international trainees), eight PhD students and five MS students. In addition he has served as a member of several other students' advisory committees. He has served on the Editorial Board for the American Journal of Veterinary Research and Theriogenology publications. Dr. Wagner served on the Scientific Advisory Board of the Morris Animal Foundation (1977-81, Chair 1980-81)

In organized veterinary medicine, Dr. Wagner has served on the Council on Education of the AVMA and as Chair of the COE in 1991. He also was the ACT representative on the Advisory Board on Veterinary Specialties, 1971-1979. Dr. Wagner is a Life Member of the Conference of Research Workers in Animal Diseases (CRWAD).

2012 CRWAD - Keynote Speaker - Immunology Section
AAVI Distinguished Veterinary Immunologist Award

Dr. Michael P. Murtaugh, Department of Veterinary & Biomedical Sciences, College of Veterinary Medicine, University of Minnesota, St. Paul, MN

Abstract No. 124 - Title: Moving Swine Immunology Forward Through Molecular and Vaccine Technology

Monday, December 3, 1:30 PM - Salons F/G/H, 5th Floor

Dr. Michael P. Murtaugh's scientific journey began at the University of Notre Dame, where George Craig in the Department of Biology channeled his love of science in the direction of entomology. After graduating with a BS degree in biology in 1973, he joined the Peace Corps and served two years in Maracay, Venezuela, at the Centro Nacional de Investigaciones Agropecuarias, where he carried out research on non-insecticidal methods of pest control for yield improvement in maize. During this time, he amassed a collection of about 12,000 insects, and traveled throughout Venezuela and South America, thus developing an appreciation for the evolutionary diversity of insects and the cultural diversity of humans. He entered the entomology PhD program at Ohio State University in 1976 and left in 1980 with a dissertation on the regulation of egg laying in the house cricket, *Acheta domestica*, under the guidance of David Denlinger, who was recently elected to the National Academy of Science, two children, and awareness that a deeper knowledge of cell biology was needed to understand biological regulatory mechanisms. A postdoc appointment in the Department of Medicine, University of Texas Medical Center at Houston, filled this gap in knowledge. Working with Peter J.A. Davies, he became an expert in the regulation of transglutaminase expression in macrophages and dipped a toe into molecular biology. He joined the faculty of the Department of Veterinary Pathobiology at the University of Minnesota in 1985 as the token molecular biologist. With no veterinary background whatsoever, his chair, Victor Perman, asked only that he develop a research program that had something to do with animal health. This sage advice, combined with an energetic and ambitious faculty group in swine medicine and a supportive state swine industry, led him to develop a program in molecular mechanisms of disease resistance, focused on pigs, that has guided the lab for the following quarter century.

In the late 1980's there were few reagents available to investigate porcine immunology, so the first challenge was to use the new power of recombinant DNA technology to clone, express, and purify cytokines. It was a fertile time for a molecular biologist, even one who had never done a Southern blot, in a College of Veterinary Medicine. Papers were published describing cytokine biology in swine, molecular diagnostic tools for bacterial pathogens, and collaborative research in neurobiology, pharmacology, and related topics. Studies in porcine pleuropneumonia showed that In 1990, a new viral disease of swine emerged simultaneously in North America and Europe. Porcine reproductive and respiratory syndrome virus was, and remains still, a devastating disease of swine. The lab became involved in molecular analysis and evolution of PRRSV and has made extensive basic and translational contributions to the understanding of porcine immune responses to PRRSV. He was the director of the PRRS Coordinated Agricultural Project, the first USDA program project, from 2004 to 2008 and has lectured extensively on PRRS immunology, vaccinology, and diagnostics throughout the world. Recently his lab has initiated a similar program to elucidate the immunological interaction of swine with porcine circoviruses, and has contributed to annotation of immune response genes in the porcine genome.

In addition to maintaining an active research program that provides a home to undergraduates, graduate students, postdocs, and visiting scientists, he provides community outreach with molecular biotechnology workshops for educators, professionals, and international scientists, directs the Comparative and Molecular Biosciences graduate program, and regularly reviews grants and manuscripts. His many contributions have been recognized through the CVM Pfizer Award for Research Excellence (four times) and the University of Minnesota Inventor Recognition Award in 2005, a University Innovations Award in 2011, and the Allen D. Leman Swine Conference Pijoan Lectureship in 2008.

George Washington Pugh, Jr. DVM, Ph.D
1934 – 2012 In Memoriam

Dr. George Washington Pugh Jr. DVM. Ph.D, of Ames, passed away of cancer Sunday, June 3, 2012, at Israel Family Hospice House in Ames . Dr. Pugh was an internationally recognized research leader in infectious animal diseases. In 1961, he became the first black person to receive a license to practice veterinary medicine in Georgia, and the first black research scientists ever hired or retained by the United States Department of Agriculture (Agricultural Research Service Division). An author and contributor to hundreds of research articles, his breakthrough work in immunity, immunogenicity and vaccines, brucellosis, pink eye, and other diseases led to substantial research advances. Throughout his career, he helped teach hundreds of veterinary and graduate students, launching them into careers around the world in microbiology and veterinary science and helping the National Animal Disease Center (NADC) in Ames to international prominence.

Dr. Pugh was born April 15, 1934 in Hurtsboro, Alabama, the second son of the Reverend George W. Pugh Sr. and Cathel Dix. After graduation from high school in 1952, he enlisted in the U.S. Army and served with distinction in Korea. After completing his military service, he became active in the civil rights movement, participating in the Montgomery bus boycott and sit-ins to protest segregation. Using the GI Bill, he enrolled in Tuskegee Institute in Tuskegee, Alabama, where he earned a DVM in 1961. After moving to Ames to work for the NADC, he began work on his doctorate and earned a Ph.D in microbiology from Iowa State University in 1971. His scientific achievements resulted in several professional distinctions, including: membership in the veterinary honor society Phi Zeta (1969), the scientific research society Sigma Xi (1971), appointment as an honorary Kentucky Colonel, and an appointment as a diplomat of the American College of Veterinary Microbiologists. Dr. Pugh retired from the NADC in 1996 and became active in the Ames community. He spent his time gardening and participating in the North Grand Farmers Market where he always ensured that everyone went away with more vegetables and information than expected.

He is survived by his wife of 49 years, Jeanette Pugh, of Ames; children, David (Rose) Pugh, of Jacksonville, Fla., Deborah Pugh, of Ames, Joseph (Melissa) Pugh, of Stillwater, Minn., Jeanne Pugh, of Woodbury, Minn.; and three grandchildren, Malcolm, Alexis, and Andrea. He maintained close ties with his extended family and is also survived by his aunt, Annie Lou Anthony, of Orlando, Florida; his brother, the Reverend Andrew (Louise) Pugh, of Alabama; his life-long friend and late sister's husband, Nathaniel Dubose, of Los Angeles; and his mother-in-law, Rosa De Souza, of Washington, D.C.

He was preceded in death by his parents; sister, Elizabeth Dubose, of Los Angeles; and his first wife, Adrienne De Souza Pugh, of Washington, D.C.

2012 Mark Gearhart Memorial Graduate Student Award

Title: Nasal shedding of Equine Herpesvirus-1 from horses in an outbreak of Equine Herpes Myeloencephalopathy in Western Canada

Brandy A. Burgess[1,2], N Tokateloff[1], K Poirier[1], S Manning[1], K Lohmann[1], DP Lunn[2], SB Hussey[2], PS Morley[2].

1. Department of Large Animal Clinical Sciences, Western College of Veterinary Medicine, University of Saskatchewan, Saskatoon, Saskatchewan, Canada. 2. Animal Population Health Institute, Colorado State University, Fort Collins, CO.

Recently, Equine Herpes Myeloencephalopathy (EHM) was described as a "potentially emerging disease" by the USDA. Absence of information regarding shedding in horses with naturally occurring disease, and knowledge that latent carriage complicates management, makes development of objective quarantine recommendations for managing outbreaks difficult. Objectives of this report were to describe an outbreak of EHM in western Canada during the spring of 2008 and evaluate nasal shedding duration of Equine Herpesvirus – 1 (EHV-1) in horses affected with EHM during this outbreak.

All horses on affected premises were monitored. Those horses developing EHM were sampled in a longitudinal outbreak investigation. Nasal swabs were collected daily from 16 of 20 horses affected by EHM. A qPCR was performed on 98 of 246 nasal swab samples to determine nasal shedding duration. Historical and clinical information was analyzed to evaluate potential risk factors for developing EHM and duration of shedding during this outbreak.

The last day shedding was detected in any horse was Disease Day 9. EHV-1 was detected in two-thirds of horses tested on Disease Days 0–3. The amount of EHV-1 DNA found in nasal swabs varied markedly and was not associated with disease severity or age. The odds of developing EHM were greater for febrile horses (OR = 20.3; 95% CI 3.4–390.3; P = .01) as well as for horses attending the riding clinic (OR = 4.1; 95% CI 0.84–21.65; P = .08).

Based on these findings, in the absence of laboratory testing, we recommend biosecurity measures be implemented when managing EHM cases for a minimum of 14 days beyond the onset of clinical signs. This report illustrates that animal managers cannot rely on the severity of clinical signs to predict the duration of EHV-1 shedding.

PROGRAM

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CEVA ANIMAL HEALTH

The CRWAD Conference is supported by the National Research Initiative (NIFA) of the USDA Cooperative State Research, Education and Economics National Institute of Food and Agriculture Award No. 2010-65119-20597 .

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2012 CRWAD Keynote Speakers and Titles

Bacterial Pathogenesis Section – Dr. Yasuko Rikihisa
Professor, Department of Veterinary Biosciences, The Ohio State University, Columbus, Ohio
Monday, December 3, 10:45 AM - Avenue Ballroom, 4th Floor
No. 010 - Title – Roles of type IV secretion system in obligatory intracellular infection.

Biosafety and Biosecurity Section – Dr. Alexei D. Zaberezhny
Professor, Head of Laboratory, D. I. Ivanovski Institute of Virology, Moscow, Russia.
Monday, December 3, 3:00 PM - Denver/Houston Room, 5th Floor
No. 028 - Title - African Swine Fever: Current Situation and Control Strategy.

Companion Animal Epidemiology, Epidemiology & Animal Health Economics, and Food & Environmental Safety Sections – Marcus G. Doherr
Veterinary Public Health-Institute (VPHI), University of Bern, Liebefeld, Switzerland
Tuesday, December 4, 8:00 AM - Salons A/B/C/D, 5th Floor
No. 031 - Title - From licking stamps to clicking buttons – moving from conventional questionnaires to online surveys

Gastroenteric Diseases Section – Dr. Srinand Sreevatsan
College of Veterinary Medicine, University of Minnesota, St. Paul, MN
Monday, December 3, 8:45 AM – Michigan/Michigan State Room, 6th Floor
No. 103 - Title - Unveiling the mysteries of iron regulation in *Mycobacterium avium* subspecies *paratuberculosis*

Immunology Section – Distinguished Veterinary Immunologist – Dr. Michael P. Murtaugh
Department of Veterinary & Biomedical Sciences, CVM, University of Minnesota, St. Paul, MN
Monday, December 3, 1:30 PM - Salons F/G/H, 5th Floor
No. 124 - Title - Moving Swine Immunology Forward Through Molecular and Vaccine Technology

Respiratory Diseases - Dr. Anthony Confer
Oklahoma State University, Stillwater, OK
Monday, December 3, 3:45 PM - Indiana/Iowa Room, 6th Floor
No. 156 - Title - *Mannheimia haemolytica* Immunity: Are we there yet?

Vector-Borne and Parasitic Diseases – Dr. Robert A. Heinzen
Department of Health & Human Services, National Institutes of Health Institute of Allergy and Infectious Diseases, Hamilton, MT
Monday, December 3, 10:00 AM - Denver/Houston Room, 5th Floor
No. 171 - Title - Recent advances in research of the Q fever bacterium, Coxiella burnetii

Viral Pathogenesis Section – Dr. Daniel R. Perez
Veterinary Medicine, University of Maryland, College Park, MD
Tuesday, December 4, 10:45 AM - Los Angeles/Miami/Scottsdale, 5th Floor
No. 202 - Title – Of Men, Pigs, Birds and…Flu

2012 CRWAD - Keynote Speaker - Bacterial Pathogenesis Section

Yasuko Rikihisa, PhD
Professor, Department of Veterinary Biosciences, The Ohio State University, Columbus, OH

Abstract No. 010 - Title: Roles of type IV secretion system in obligatory intracellular infection.
Monday, December 3, 10:45 AM - Avenue Ballroom, 4th Floor

Dr. Rikihisa obtained her Ph.D. from University of Tokyo, Japan, and her postdoctoral training at Harvard Medical School. She specializes in the study of vector-borne diseases that affect food and fiber-producing animals, companion animals and humans. Her research focuses on understanding how unique bacterial pathogens *Ehrlichia, Anaplasma*, and *Neorickettsia* can infect and thrive within primary host defensive white blood cells, and cause potentially fatal emerging infectious diseases. Her findings suggest that these bacteria use proteins directly secreted into host cell cytoplasm to manipulate immune-system cells in animal and human hosts, effectively creating safe havens for themselves until they can build up enough strength and numbers to cause dangerous disease. Her research group also isolated, molecularly characterized, and named several new species of this group of bacteria, and developed new diagnostic methods.

A member of Ohio State's faculty since 1986, Dr. Rikihisa was elected as a member of National Academy of Sciences in 2012 and named the university's 2011 Innovator of the Year by the Office of Research in recognition of her record of translational research. She also is a fellow of the American Association of the Advancement of Science and the American Academy of Microbiology, received the Ohio State Distinguished Scholar Award in 1999. She was a former President and Vice president of American Society for Rickettsiology, and served as USDA Grant Review Panel member and as a member of NIH study sections. She has more than 250 peer-reviewed scientific publications and 24 chapters in books and proceedings. She has nine US issued patents, and four issued foreign patents. She is an investigator in Ohio State's Center for Microbial Interface Biology, the Public Health Preparedness for Infectious Diseases program, the Molecular, Cellular and Developmental Biology program, and the Comprehensive Cancer Center.

2012 CRWAD - Keynote Speaker - Biosafety and Biosecurity Section

Dr. Alexei D. Zaberezhny
Professor, Head of Laboratory, D. I. Ivanovski Institute of Virology; and Y. R. Kovalenko All-Russian Research Institute of Experimental Veterinary Medicine, Moscow, Russia

Abstract No. 028 - Title: African Swine Fever: Current Situation and Control Strategy.
Monday, December 3, 3:00 PM - Denver/Houston Room, 5th Floor

Education:

- MS in Molecular Biology, Moscow Engineering Physics Institute, 1983
- Additional Training: Molecular Biology Training Course (8 months) at Biology Academic Center of Moscow State University, Puschino-on Oka, 1984-1985.
- Ph.D. (Candidate of Science) in Biochemistry, Y. R. Kovalenko All-Union Research Institute of Veterinary Medicine, Academy of Agricultural Sciences (USSR Academy of Agricultural Sciences), Moscow, 1988.
- Doctor of Science in Virology, D. I. Ivanovski Virology Institute, Russian Academy of Medical Science, Moscow. 2004
- Professor in Virology, 2010

Professional experience:

- Research Assistant (1983), Moscow Institute of Engineering Physics, Moscow.
- Junior Research Fellow (1983-1988).
- Senior Research Fellow (1989-1990) at the Laboratory of Molecular Biology & Biochemistry, All-Union Research Institute of Experimental Veterinary Medicine, Moscow.
- Postdoctoral Scientist (1990 - 1993) at Veterinary Medical Research Institute, College of Veterinary Medicine, Iowa State University, Ames IA.
- Postdoctoral Scientist (1993 - 1994).
- Senior Researcher (1994 - 1996) at Viral Vaccine Division, Lederle-Praxis Biologicals, a Division of American Home Products, Pearl River, NY.
- Head of Research and Development Department (1997-present) at JSC NARVAC, (at D. I. Ivanovski Virology Institute, Moscow).
- Head of Laboratory of Molecular Diagnostics (2001-2006) at D. I. Ivanovski Virology Institute, Russian Academy of Medical Science, Moscow.
- Head of Laboratory of Applied Biotechnology (2006-present) at D. I .Ivanovski Virology Institute, Russian Academy of Medical Science, Moscow.
- Member of Editorial Board of Voprosy Virusologii (Problems of Virology) since 2006
- Deputy Editor-in-Chief of Voprosy Virusologii (Problems of Virology) since 2008
- Deputy Director, Y. R. Kovalenko All-Russian Research Institute of Experimental Veterinary Medicine, (Russian Academy of Agricultural Sciences), Moscow, since 2012

Honors and awards:

- Silver medal of All-Union Exhibition of People's Economic Achievements, 1987
- Member of USSR Society for Biochemistry 1983-1990.
- Full Member of American Society for Virology since 1995.
- Grant of Regional Development for Scientists: 2008-2009

2012 CRWAD - Keynote Speaker for the Companion Animal Epidemiology Section, Epidemiology & Animal Economics Section and Food & Environmental Safety Section

Dr. Marcus G. Doherr, Diplomat ECVPH
Department Clinical Research & VPH, Vetsuisse Faculty
Veterinary Public Health Institute (VPHI), University of Bern
Liebefeld (BE), Switzerland

Abstract No. 031 - Title: From licking stamps to clicking buttons – moving from conventional questionnaires to online surveys.

Tuesday, December 4, 8:00 AM - Salons A/B/C/D, 5th Floor

My scientific career started with attending the Hannover (Germany) veterinary school to graduate as a veterinaran in 1989. After completing a research thesis (DVM) on ELISA development and validation for Babesia bovis and a short period in university research an administration I received a Fulbright scholarship and was accepted into the University of California, Davis, Epidemiology Graduate Group. Under the supervision of Prof. Tim Carpenter I completed a PhD in Epidemiology with a research project on Corynebacterium pseudertuberculosis epidemiology in horses in California. Since 1998 I am working as a veterinary epidemiologist in Switzerland, first for the Swiss Federal Veterinary Authorities and then at the Vetsuisse Faculty, University of Bern. My main interests are in designing, implementing and interpreting population based montitoring and surveillance programs. In addition I am responsible for the epidemiology teaching and consulting at the Bern Veterinary faculty, and contributed to over 170 peer-reviewed research publications and several book chapters.

In addition to holding a DVM and PhD degree I since 2002 am Diplomate of the European College of Veterinary Public Health (ECVPH), and one of the associate editors of Preventive Veterinary Medicine. From 2009 to 2012 I served as a scientific expert in the standing Animal Health and Animal Welfare (AHAW) Panel for the European Food Safety Authority (EFSA).

2012 CRWAD - Keynote Speaker for the Gastroenteric Diseases Section

Srinand Sreevatsan, MVSc, MPH, PhD
Veterinary Population Medicine and Veterinary Biomedical Sciences Departments,
College of Veterinary Medicine, University of Minnesota, St. Paul, MN

Abstract No. 103 - Title: Unveiling the mysteries of iron regulation in *Mycobacterium avium* subspecies *paratuberculosis*

Monday, December 3, 8:45 AM – Michigan/Michigan State Room, 6th Floor

Degrees:
B.V.Sc., University of Agricultural Sciences, Bangalore, India (1986)
M.V.Sc, University of Agricultural Sciences, Bangalore, India (1988)
M.P.H., University of Minnesota, Minneapolis, Minnesota (1991)
Ph.D., University of Minnesota, St. Paul, Minnesota (1995)

Dr. Sreevatsan is a Professor of Infectious Disease at the College of Veterinary Medicine, University of Minnesota. The principal focus of his laboratory is to define the molecular mechanisms by which bacteria and viruses efface, enter, and establish infection in their hosts. His interests surround several issues in microbe-host interactions with specific emphasis on the evolution of the pathogen and it's adaptation to hosts. The translational aspect of these investigations is in the development of improved diagnostic tests and methods for microbial characterization and identification, as well as studies into new generations of antimicrobial vaccines and therapeutics. A second focus in his laboratory is in the improvement of currently available diagnostic tools. As a result some investigations use state-of-the-art molecular methods including the design of novel high affinity ligands and sensitive back-end detection methods. These are coupled with classical and modified extraction protocols to improve recovery of agents of interest for accurate diagnostics. Dr. Sreevatsan is currently investigating the molecular diversity and pathogenesis of mycobacterial diseases, microbial population structure and functioning in pathogen induced environments, influenza virus ecology and evolution, and developing high affinity ligands to investigate pathogenesis and new therapeutic modalities for infectious diseases.

2012 CRWAD - Keynote Speaker - Respiratory Diseases Section

Dr. Anthony W. Confer
Regents Professor and Sitlington Endowed Chair for Food Animal Research, Department of Veterinary Pathobiology, Oklahoma State University, Center for Veterinary Health Sciences.

Abstract No. 156 - Title: *Mannheimia haemolytica* Immunity: Are we there yet?

Monday, December 3, 3:45 PM - Indiana/Iowa Room, 6th Floor

Anthony W. Confer — Regents Professor and Sitlington Endowed Chair for Food Animal Research, Department of Veterinary Pathobiology, Oklahoma State University, Center for Veterinary Health Sciences. Dr. Confer received the DVM form Oklahoma State University, 1972, M.S. in Pathology from Ohio State University, 1974, and Ph.D. in Microbiology from University of Missouri, 1978. He is a Diplomate, American College of Veterinary Pathologists. He served as Captain, Veterinary Corp, US Air Force from 1974-1976 at the Armed Forces Institute of Pathology. Dr. Confer joined the faculty of Oklahoma State University in 1981. Since that time, his major research interests and focus have been in bovine respiratory disease pathogenesis and immunity especially related to *Mannheimia haemolytica* and *Pasteurella multocida* infections. His laboratory in conjunction with Dr. Sahlu Ayalew is currently focused on the role of outer membrane proteins in stimulating immunity to *M. haemolytica*. He is author or co-author of 205 refereed scientific publications, 121 published abstracts, 13 book chapters, 14 continuing education publications, and four veterinary medical education manuscripts. As Principal Investigator, Dr. Confer has obtained >$8,000,000 in extramural research funding. He and Dr. Ayalew hold two US Patents. Dr. Confer served as a department head from 1986-1999 and again from 2004-2008 and as Associate Dean for Research from 1999-2001. He received the Norden Distinguished Teacher Award in 1987 & 2002, Pfizer Award for Research Excellence in 1988 & 2011, OSU Regents Distinguished Teaching Award in 2008, and the Oklahoma State University Eminent Faculty Award in 2003. The OSU College of Veterinary Medicine recognized him as a Distinguished Alumnus in 2009. Dr. Confer has been a CRWAD Member since 1982.

2012 CRWAD - Keynote Speaker - Vector-Borne and Parasitic Diseases Section

Dr. Robert A. Heinzen, Senior Investigator and Section Head
Coxiella *Pathogenesis Section , Laboratory of Intracellular Parasites*
Department of Health & Human Services, National Institutes of Health Institute of Allergy and Infectious Diseases, Hamilton, MT

Abstract No. 171 - Title: Recent advances in research of the Q fever bacterium, Coxiella burnetii

Monday, December 3, 10:00 AM - Denver/Houston Room, 5th Floor

Dr. Robert A. Heinzen received his Ph.D. in Microbiology from Washington State University in 1991. After completing an Intramural Research Training Award fellowship in the Laboratory of Intracellular Parasites at the NIH in 1996, Dr. Heinzen joined the faculty of the University of Wyoming as an assistant professor of Molecular Biology where he was awarded tenure and promoted to associate professor in 2002. Dr. Heinzen was recruited to the NIH in 2003 as Head of the new *Coxiella* Pathogenesis Section in the Laboratory of Intracellular Parasites. He was promoted to Senior Investigator with tenure in 2010. Dr. Heinzen has served on the executive council for the American Society Rickettsiology. and is a past recipient of extramural NIH funding for his rickettsial work. He has served on numerous grant study sections and journal reviews and is internationally recognized for his studies on *Coxiella* and *Rickettsia* pathogenesis.

2012 CRWAD - Keynote Speaker - Viral Pathogenesis Section

Dr. Daniel R. Perez
Associate Professor, Veterinary Medicine, University of Maryland, College Park, MD

Abstract No. 202 - Title: Of Men, Pigs, Birds and…Flu

Tuesday, December 4, 10:45 AM - Los Angeles/Miami/Scottsdale, 5th Floor

Daniel obtained his BSc in Biochemistry from the National University of Cordoba, Argentina in 1989 and completed his PhD in Molecular Virology in the Department of Veterinary and Biomedical Sciences at the University of Nebraska, Lincoln, Nebraska in 1995. In March 2000, Dr. Perez joined the laboratory of Dr. Robert Webster at St. Jude Children's Research Hospital where he worked on biological and epidemiological aspects of avian influenza viruses. Currently, Daniel is Associate Professor in the Department of Veterinary Medicine at the University of Maryland, College Park. Daniel has been studying virus-virus and virus-host protein interactions of influenza virus and bovine viral diarrhea virus. His current interests include the interspecies transmission, pathogenesis, and evolution of avian influenza viruses and the role of cross-protective immunity in the spread of highly pathogenic avian influenza viruses to other birds and mammals.

Among Dr. Perez's major scientific contributions has been the participation in the development of the first plasmid-based reverse genetics system for influenza, which allows the complete manipulation of the influenza genome. Such strategy has proven instrumental in the preparation of vaccines for pandemic preparedness, particularly against the current H5N1 and H9N2 viruses.

Using reverse genetics and classical virology, Dr. Perez studies are aimed at better characterizing intermediates hosts as contributors in the adaptation, spread, and perpetuation of novel avian influenza variants that can be transmitted to other poultry and to mammals, including humans.

Dr. Perez is currently heading a major research project with the collaboration of 17 other institutions across the US and funded by the USDA. Currently in its last year, this 6 year project with over $10 million dollars in funding entitled "Prevention and Control of Avian Influenza in the US" is the largest granted by the USDA to combat a single disease. Dr. Perez's lab is also a member of the NIAID-funded Center for Research on Influenza Pathogenesis along with Mount Sinai School of Medicine, Erasmus Medical Center, University of Wisconsin-Madison and other research partners.

2012 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 – 5th Floor Foyer Registration Booth
Sunday, Dec. 2, 10 AM - 5:30 PM
Monday, Dec. 3, 7:00 AM - Noon, 2 - 5 PM
Tuesday, Dec. 4, 8 - 11 AM

CRWAD Researchers Reception and Poster Session I - Grand Ballroom Salon III - 7th Floor
(Poster I Sections listed inside front cover)
Sunday, Dec. 2, 6-8 PM - Reception
Poster Session I Set-up - 4 PM (Section Posters are listed in the Summary Table)
Remove posters by 10:00 AM Monday
First Poster Session - 6:30-8 PM
All Attendees are Welcome. Please join us. Casual wear recommended.

CRWAD Poster Session II - Grand Ballroom Salon III - 7th Floor
Monday, Dec. 3 - 5:00 PM - 6:30 PM
Poster Session II Set-up - 12:00 PM (Section Posters are listed inside the front cover)
Remove posters immediately upon completion of Poster Session II.

CRWAD Student Reception
5:00 PM – 5:45 PM, Salon II Room, 7th 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. 2, **Board Meeting**
8 AM - 12 PM – Los Angeles Room - 5th Floor
Monday, Dec. 3, **Business Meeting and Luncheon**
11:30 AM - 1PM - Buca di Beppo Restaurant
For more information contact Gina Pighetti

American College of Veterinary Microbiologists (ACVM)
Examination - Denver/Houston Room - 5th Floor
Friday, Nov. 30, 8 AM - 8 PM
Saturday, Dec. 1, 8 AM - 9 PM

Examination - Kansas City Room – 5th Floor
Saturday, Dec. 1, 8 AM – 1 PM

Sunday, Dec. 2, Denver/Houston Room - 5th 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 Amelia Woolums.

2012 CRWAD AND SATELLITE MEETINGS
(Alphabetically listed)

Animal Health Research Reviews (AHRR) Board Meeting
Tuesday, Dec. 4, 7 - 9:30 AM – Sheffield Room - 4th Floor
Section Editors and Editorial Board joint meeting.
For more information contact Bill Stich, Editor in Chief

AVEPM Schwabe Symposium - "Making sense of the world around us - Methods in observational research"
A Symposium Honoring the Legacy of Dr. Ian Dohoo
(Association for Veterinary Epidemiology and Preventive Medicine)
Sunday, Dec. 2, 11:30 PM - 5 PM, Chicago Ballroom Salon E/F/G/H Room - 5th Floor

Formal presentation to Dr. Dohoo will be during CRWAD Business Meeting, Tues. Dec. 4
11:45 AM - 12:30 PM, Chicago Ballroom A/B/C/D, 5th Floor
For more information contact H. Morgan Scott and Jan Sargeant.

AVEPM Business Meeting – Buffet Luncheon - Members only
(Association for Veterinary Epidemiology and Preventive Medicine)
Monday, Dec. 3, 11:30 AM – 1:30 PM - Northwestern/Ohio/Purdue Room - 6th Floor
For more information contact Morgan Scott

Brucellosis Research Group Meeting
Saturday, Dec. 1, Registration and poster assembly, 7:00 – 8:00 AM, Salons A/B/C/D - 5th Floor
Saturday, Dec. 1, 8:00 AM – 5:00 PM, Salons A/B/C/D - 5th Floor
Sunday, Dec. 2, 7:30 AM – 5:00 PM, Salons A/B/C/D - 5th Floor
For more information contact Sue Hagius - cell phone: 225-931-1132

CRWAD Council Meeting
Saturday, Dec. 1, 5:30 PM - 9 PM - Great America Room - 6th Floor

CRWAD Business Meeting
Tuesday, Dec. 4, 11:45 AM - 12:30 PM - Chicago Ballroom A/B/C/D - 5th 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. 1, 5:30 – 6:00 PM, Great America Room - 6th Floor

Distinguished Veterinary Immunologist Lecture by Dr. Michael P. Murtaugh
Department of Veterinary & Biomedical Sciences, CVM, University of Minnesota, St. Paul, MN
Monday, Dec. 3, 1:30 PM - Salons F/G/H, 5th Floor
Title – Moving Swine Immunology Forward Through Molecular and Vaccine Technology

Distinguished Veterinary Microbiologist is Dr. Leon N. D. Potgieter
The University of Tennessee, Knoxville, TN

2012 CRWAD AND SATELLITE MEETINGS
(Alphabetically listed)

Elsevier Meetings

Editorial Board of Meeting #1
Sunday, Dec. 2, 7:00 AM - 9 AM - Lincolnshire Room, 6th floor 2011

Editorial Board of Meeting # 2
Monday, Dec. 3, 7:30 AM - 9 AM - Minnesota Room, 6th Floor

Exhibitors - Sunday - Monday, Dec. 2-3, 5th Floor Foyer
7:30 AM – 5 PM (exhibits close Monday, Dec. 3, 5 PM)
Elsevier BV
Kingfisher Biotech, Inc.
List Biological Laboratories
MabTech, Inc.
PerkinElmer
Seppic, Inc.
Tetracore, Inc.

Integrated Special Emphasis Project
Minimizing Antibiotic Resistance Transmission throughout the Food Chain
Saturday, December 1
11:00 AM - 5:00 PM, Northwestern/Ohio Room – 6th Floor

Sunday - December 2
7:00 AM - 11:00 AM, Northwestern/Ohio Room – 6th Floor
For more information contact H. Morgan Scott, Kansas State University: 785-532-4602

Members of the Pilot Sampling Program for Antimicrobial Resistance
Tuesday, Dec. 4, 1:30 PM – 5:00 PM - Sheffield Room, 4th Floor
For more information contact Mary Torrence at Mary.Torrence@ars.usda.gov

Mycobacterial Diseases of Animals Multistate Initiative
Sunday, December 2, 1PM – 6PM – Northwestern/Ohio Room – 6th Floor
For information contact: Ken Olson, JDIP Outreach Coordinator: 1-630-237-496; keolson@prodigy.net

NC-1180 Respiratory Diseases of Poultry Committee Meeting
Sunday, December 2, 8 AM - 5 PM - Michigan/Michigan State Room Room - 6th Floor
For more information contact Y. M. Saif (saif.1@osu.edu)

NC-1202 (formerly 1041) Enteric Diseases of Food Animals: Enhanced Prevention, Control and Food Safety
Saturday, Dec. 1, 8 AM - 5 PM – Miami Room - 5th Floor
Sunday, Dec. 2, 8 AM - 12 PM - Miami Room - 5th Floor
Attendance is by invitation only.
For more information contact Qijing Zhang. (Zhang123@iastate.edu)

2012 CRWAD AND SATELLITE MEETINGS
(Alphabetically listed)

NC-229 Porcine Reproductive and Respiratory Syndrome Virus Meeting (PRRS)

Sunday, December 2, 1 PM - 5PM, Denver/Houston/Kansas City Room
Title: Detection and Control of Porcine Reproductive and Respiratory Syndrome Virus and Emerging Viral Diseases of Swine

Monday, Dec. 3-4, Oral Abstracts will present in the CRWAD Viral Pathogenesis Section; Respiratory Diseases Section
Attendance is open. For more information contact Jane Christopher-Hennings

USDA-National Institute of Food and Agriculture (NIFA)

Agriculture and Food Research Initiative (AFRI)
Animal Health and Well-being Project Director Workshop
Open Workshop for AFRI animal health and welfare awardees AND other interested individuals.

Saturday, Dec. 1, 7:00 AM - 5 PM – Salons E/F/G/H Room - 5th Floor
Poster Boards in Salon E

For more information, please contact: Davida Tengey (dtengey@nifa.usda.gov); Margo Holland (mholland@nifa.usda.gov); or Peter Johnson (pjohnson@nifa.usda.gov)

CRWAD ABSTRACTS AVAILABLE AT:
The On-Line Meeting Planner and Itinerary Builder

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

Making Sense of the World Around Us - Methods in Observational Research – A Symposium Honoring the Professional Legacy of Dr. Ian Dohoo –

The Association of Veterinary Epidemiology and Preventive Medicine (AVEPM) is pleased to announce the program for the 2012 Schwabe Symposium honoring the professional achievements of Dr. Ian Dohoo. The symposium will be held in Chicago on **Sunday, Dec 2, 2012**, at the Chicago Marriott, Downtown Magnificent Mile, Chicago, Illinois, just prior to the opening of the Congress of Research Workers in Animal Diseases. There is no registration fee for the symposium, and all are welcome to attend.

11:30 pm Light buffet lunch for attendees
12:30 pm Welcome and Introductory remarks from Hollis Erb

12:40 pm *The Framework - Making causal inferences from observational studies*
Wayne Martin, Professor Emeritus, Ontario Veterinary College
1:15 pm *The Data - Sources and validation*
Ulf Emanuelson, Professor, Swedish University of Agricultural Sciences
1:50 pm *The Analysis - Where are we and where are we going?*
Henrik Stryhn, Associate Professor of Biostatistics, Atlantic Veterinary College

2:25 pm Break and Refreshments

2:55 pm *The Synthesis - Meta-analysis of observational research*
Annette O'Connor, Professor, Iowa State University, College of Veterinary Medicine
3:30 pm *The reporting - Guidelines for observational studies in veterinary medicine*
Jan Sargeant, Professor, Ontario Veterinary College

Keynote address:
4:05 pm *Bias - Is it a problem and what should we do?*
Ian Dohoo, Professor Emeritus, Atlantic Veterinary College, University of Prince Edward Island

4:50 pm Closing comments
6:00 – 8:00 pm CRWAD Researchers Reception and Poster Session I

The Calvin W. Schwabe Award is presented annually by the AVEPM to honor lifetime achievement in veterinary epidemiology and preventive medicine. The 2012 honoree is:

Ian Dohoo DVM, PhD, FCAHS

Dr. Ian Dohoo is a Professor Emeritus of epidemiology at the University of Prince Edward Island and the immediate past-Director of the Centre for Veterinary Epidemiological Research (www.upei.ca/cver). His extensive publication and graduate student supervision records, combined with authorship of the leading graduate level text in the field (Veterinary Epidemiologic Research – www.upei.ca/ver), have established his reputation as a leading international figure in veterinary epidemiology and population-based health research. He is recognized as an excellent teacher both locally and internationally and is frequently involved in the delivery of high level post-graduate courses around the world. He has served as President of the Canadian Association of Veterinary Epidemiology and Preventive Medicine and has received numerous teaching and research awards. In 2005 he was one of four veterinarians in Canada elected as an inaugural Fellow of the Canadian Academy of Health Sciences. In 2008 he was awarded an honorary Veterinary Medical Doctorate by the Swedish University of Agricultural Sciences and in 2012, an honorary Doctor of Science by the University of Guelph.

2012 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 (2nd floor) is available on Sunday, Dec. 2 - Monday, Dec. 3

POSTER INFORMATION - Poster Sessions I & II - Grand Ballroom III, 7th Floor
SUNDAY POSTER PRESENTERS: December 4, 6:30 - 8:00 PM.
Poster boards will be available for poster assembly by 4 PM Sunday. Posters for the Bacterial Pathogenesis, Biosafety and Biosecurity, Companion Animal Epidemiology, Epidemiology and Animal Health Economics, and Gastroenteric Diseases Sections will be presented Sunday from 6:30-8:00 PM. Please remove your posters by 10:00 AM Monday.

MONDAY POSTER PRESENTERS: December 5, 5:00 - 6:30 PM
Poster boards will be available for poster assembly by noon Monday. Posters for the Food and Environmental Safety, Immunology, Respiratory, 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 and Sunday, Dec. 1-2, 8 AM - 5:00 PM - Brucellosis Research Meeting
Saturday, Dec. 1, 8AM - 5:00 PM - NC1202 Enteric Diseases of Food Animals
Sunday, Dec. 1, 8AM - 12:00 PM - NC1202 Enteric Diseases of Food Animals
Saturday, Dec. 1, 7 AM - 5:00 PM - USDA-NIFA-AFRI Project Director Workshop
Sunday - Dec. 4, 11:30 AM - 5 PM - AVEPM Symposium Program - Open Attendance
Sunday, Dec. 2, 1 PM - 5:00 PM - NC229 PRRSV Meeting
Monday, Dec. 3, 8 AM - 5 PM - NC229 scientific abstracts for PRSSV and Viral SIV/PCV2/Other
Tuesday, Dec. 4, 8 AM - 11:30 AM - NC229 scientific abstracts for PRSSV and Viral SIV/PCV2/Other

CRWAD Meeting Begins Sunday (evening):

Notice: Section meeting rooms are listed inside front cover
Sunday - Dec. 2 - 6:00-8:00 PM - Kick-Off CRWAD Reception and Poster Session I
Monday - Dec. 3, 8:00 AM - CRWAD Sections Oral Presentations begin in eight separate rooms
Tuesday - Dec. 4, 8:00 AM - CRWAD Sections Oral Presentations begin in six rooms
Tuesday – Dec. 6, 5:00 PM – 6:30 PM – Poster Session II

Time	Oral #	Section	Monday By-The-Day Title
8:00 AM	1	BACTERIAL PATHOGENESIS	Inhibition of Pseudomonas aeruginosa biofilm formation on a biological wound dressing
8:00 AM	41	EPIDEMIOLOGY AND ANIMAL HEALTH ECONOMICS	Prioritization of zoonoses in North America: A public perspective
8:00 AM	72	FOOD AND ENVIRONMENTAL SAFETY	Herd prevalence and geographic distribution ofCoxiella burnetii in cattle bulk tank milk samples in Indiana
8:00 AM	115	IMMUNOLOGY	Bovine tuberculosis research: Immune mechanisms relevant to biomedical applications
8:00 AM	137	RESPIRATORY DISEASES	Comparing Influenza A virus isolation from oral fluid and nasal swabs in IAV inoculated pigs
8:00 AM	165	VECTOR-BORNE AND PARASITIC DISEASES	Targeted and Random Mutagenesis ofEhrlichia chaffeensisfor the Identification of Genes Required forIn vivoInfection
8:00 AM	175	VIRAL PATHOGENESIS	A novel small structural protein ORF5a is essential for porcine reproductive and respiratory syndrome virus production
8:15 AM	2	BACTERIAL PATHOGENESIS	The role of exopolyphosphatase/ guanosine pentaphosphate phosphohydrolase (ppx/gppa) enzymes ofcampylobacter jejuni
8:15 AM	42	EPIDEMIOLOGY AND ANIMAL HEALTH ECONOMICS	Prioritization of Zoonoses in North America: Animal and human health professionals' perspective
8:15 AM	73	FOOD AND ENVIRONMENTAL SAFETY	Prevalence, distribution, and diversity of Salmonella subtypes on Michigan dairy farms in 2000-2001 and 2009.
8:15 AM	138	RESPIRATORY DISEASES	Comparing Influenza A virus detection in oral fluid and nasal swabs by a rapid antigen detection kit in IAV inoculated pigs
8:15 AM	166	VECTOR-BORNE AND PARASITIC DISEASES	Exploratory spatial data analysis of human Lyme disease cases in Texas between 2000 and 2010
8:15 AM	176	VIRAL PATHOGENESIS	Virion packaging of multiple cleavage isoforms of porcine reproductive and respiratory syndrome virus nonstructural protein 2
8:30 AM	3	BACTERIAL PATHOGENESIS	Campylobacter jejuni isolates from calves have A, B and C lipooligosaccharide (LOS) biosynthetic locus classes similar to human Guillain Barre syndrome associated strains
8:30 AM	43	EPIDEMIOLOGY AND ANIMAL HEALTH ECONOMICS	Methicillin ResistantStaphlylococcus aureusin Dairy Farms - Is there a need to worry?
8:30 AM	74	FOOD AND ENVIRONMENTAL SAFETY	Salmonella enterica in lymph nodes of cull and fed cattle at harvest
8:30 AM	116	IMMUNOLOGY	The role of bovine γδ T cells and their WC1 co-receptor in interacting with bacterial pathogens and promoting vaccine efficacy.
8:30 AM	139	RESPIRATORY DISEASES	Comparing Influenza A virus detection in oral fluid and nasal swabs by RT-PCR in IAV inoculated pigs

Time	Oral #	Section	Monday By-The-Day Title
8:30 AM	167	VECTOR-BORNE AND PARASITIC DISEASES	Transplacental transmission of a human isolate ofAnaplasma phagocytophilum in an experimentally infected sheep.
8:30 AM	177	VIRAL PATHOGENESIS	Host cell gene expressions and cell cycle progression regulated by PRRS virus Nsp11 protein
8:45 AM	4	BACTERIAL PATHOGENESIS	Distribution of virulence genes in Canadian Haemophilus parasuis strains
8:45 AM	44	EPIDEMIOLOGY AND ANIMAL HEALTH ECONOMICS	Non-tuberculous mycobacteria in the pastoral ecosystems of Uganda: \One health
8:45 AM	75	FOOD AND ENVIRONMENTAL SAFETY	Salmonella recovery from the peripheral lymph nodes following intradermal administration and evaluation of a commercially-availableSalmonella vaccine
8:45 AM	103	GASTROENTERIC DISEASES	Gastroenteric Diseases Section Keynote: Unveiling the mysteries of iron regulation inMycobacterium avium subspecies paratuberculosis
8:45 AM	117	IMMUNOLOGY	Characterization of the antigen-specific γδ T cell response following virulent Mycobacterium bovis infection in cattle
8:45 AM	140	RESPIRATORY DISEASES	Kinetics of influenza A virus (IAV) anti-nucleoprotein antibody (IgM, IgA, IgG) in serum and oral fluid specimens
8:45 AM	168	VECTOR-BORNE AND PARASITIC DISEASES	Inactivation of bacteria in milk using a flow-through UV-light treatment system.
8:45 AM	178	VIRAL PATHOGENESIS	Suppression of host gene expression by nsp1β protein of porcine reproductive and respiratory syndrome virus
9:00 AM	5	BACTERIAL PATHOGENESIS	Evaluation of invasion by nonpathogenicSalmonella entericaserovar Kentucky in poultry intestinal epithelia cells.
9:00 AM	45	EPIDEMIOLOGY AND ANIMAL HEALTH ECONOMICS	Comparative study of the prevalence of brucellosis in cattle, goats and humans from farms in southwestern Uganda
9:00 AM	76	FOOD AND ENVIRONMENTAL SAFETY	Sub-optimal thermal environment is associated with Salmonella shedding in swine.
9:00 AM	118	IMMUNOLOGY	WC1 functions as a pattern recognition receptor and co-receptor for γδ T cells
9:00 AM	141	RESPIRATORY DISEASES	Natural killer T cell specific adjuvants potentiates cell-mediated immunity in the pig lungs to an inactivated bivalent swine influenza H1N1 and H3N2 virus vaccine
9:00 AM	169	VECTOR-BORNE AND PARASITIC DISEASES	Temporal and spatial distribution of borreliosis, ehrlichiosis, anaplasmosis, and Rocky Mountain spotted fever in humans and dogs in Illinois from 2000-2009.
		(continued)	

Time	Oral #	Section	Monday By-The-Day Title
9:00 AM	179	VIRAL PATHOGENESIS	The PRRSV-mediated inhibition of interferon alpha production by its natural host cell occurs at the post-transcriptional level.
9:15 AM	6	BACTERIAL PATHOGENESIS	Comparative transcriptome analysis using RNA-seq reveals differences in global gene expression profiles between high-pahtogenic and low-pathogenicSalmonellaEnteritidis strains
9:15 AM	46	EPIDEMIOLOGY AND ANIMAL HEALTH ECONOMICS	Time series model for human and bovine brucellosis cases in South Korea between 2005 and 2010
9:15 AM	77	FOOD AND ENVIRONMENTAL SAFETY	A mathematical model to quantify effectiveness of cleaning as a measure to control Salmonella Typhimurium on a grower-finisher pig farm
9:15 AM	119	IMMUNOLOGY	Effector and memory T cell subsets in the response to bovine tuberculosis.
9:15 AM	142	RESPIRATORY DISEASES	Immortalized swine bone marrow epithelial cell line supports influenza virus replication.
9:15 AM	170	VECTOR-BORNE AND PARASITIC DISEASES	Evaluation of the systemic inflammatory reaction to anthelmintic treatment in ponies
9:15 AM	180	VIRAL PATHOGENESIS	Variable interference with interferon signal transduction by different PRRSV strains
10:00 AM	7	BACTERIAL PATHOGENESIS	Sequence of two plasmids from Clostridium perfringens chicken necrotic enteritis isolates and comparison with C. perfringens conjugative plasmids
10:00 AM	47	EPIDEMIOLOGY AND ANIMAL HEALTH ECONOMICS	The prevalence and spatial distribution of avian reovirus among Ontario broiler chicken flocks
10:00 AM	78	FOOD AND ENVIRONMENTAL SAFETY	False attribution: the effects of bias in probabilistic source attribution models for Salmonella infection
10:00 AM	104	GASTROENTERIC DISEASES	A novel vaccine candidate protecting cattle against diarrhea caused by enterotoxigenic Escherichia coli (ETEC) and bovine viral diarrhea virus (BVDV)
10:00 AM	120	IMMUNOLOGY	Preterm piglets are a clinically relevant model of pediatric GI disease
10:00 AM	143	RESPIRATORY DISEASES	Priming by respiratory exposure followed by intramascular boost with RNA particle vaccine in pigs in an influenza challenge model
10:00 AM	171	VECTOR-BORNE AND PARASITIC DISEASES	Vector-Borne and Parasitic Diseases Section Keynote: Recent advances in research of the Q fever bacterium, Coxiella burnetii
10:00 AM	181	VIRAL PATHOGENESIS	Identification of regulatory domain of PRRS virus nonstructural protein 1 alpha for type I interferon modulation
10:15 AM	8	BACTERIAL PATHOGENESIS	Comparative genome analysis of an avirulent and two virulent strains of avianPasteurella multocida
		(continued)	

Time	Oral #	Section	Monday By-The-Day Title
10:15 AM	48	EPIDEMIOLOGY AND ANIMAL HEALTH ECONOMICS	Prevalence, characterization, and seasonal variation ofClostridium perfringens in Ontario broiler chicken flocks.
10:15 AM	79	FOOD AND ENVIRONMENTAL SAFETY	The role of flagella in the attachment ofSalmonella enterica serovar Kentucky to broiler skin.
10:15 AM	105	GASTROENTERIC DISEASES	A genetic fusion of enterotoxins of enterotoxigenic Escherichia coli (ETEC) induced broadly antitoxin immunity against ETEC associated diarrhea
10:15 AM	144	RESPIRATORY DISEASES	A novel DNA vaccine provided efficient protection to mice against lethal dose of swine influenza virus H1N1
10:15 AM	182	VIRAL PATHOGENESIS	PRRSV nsp1β inhibits interferon signal transduction by inducing importin-α5 degradation
10:30 AM	9	BACTERIAL PATHOGENESIS	Host specificity inPasteurella multocida
10:30 AM	49	EPIDEMIOLOGY AND ANIMAL HEALTH ECONOMICS	Prevalence, seasonality, and geographical distribution of chicken anemia virus, fowl adenovirus, and infectious bursal disease virus in Ontario broiler chickens.
10:30 AM	80	FOOD AND ENVIRONMENTAL SAFETY	CTX-M-type extended spectrum β-lactamase genes inSalmonella spp. from livestock clinical diagnostic submissions in the US
10:30 AM	106	GASTROENTERIC DISEASES	Safety and immunogenicity studies of a modified heat-labile toxin (LT) and heat-stable toxin (ST) fusion protein (LTS63K/R192G/L211A-3xSTaA14Q) in a murine model
10:30 AM	121	IMMUNOLOGY	The Pig as a Model for the Study of Adipose Tissue Dysfunction in Obesity.
10:30 AM	145	RESPIRATORY DISEASES	Migration of the swine influenza virus delta-cluster hemagglutinin N-linked glycosylation site from N142 to N144 results in loss of antibody cross reactivity
10:30 AM	183	VIRAL PATHOGENESIS	The disease manifestations of two Asian highly pathogenic strains of Type 2 PRRSV
10:45 AM	10	BACTERIAL PATHOGENESIS	Bacterial Pathogenesis Section Keynote: Roles of type IV secretion system in obligatory intracellular infection.
10:45 AM	50	EPIDEMIOLOGY AND ANIMAL HEALTH ECONOMICS	The epidemiology ofBrachyspiraspecies in Ontario layer chicken flocks
10:45 AM	81	FOOD AND ENVIRONMENTAL SAFETY	Molecular characterization of the monophasic and non-motile variants ofSalmonella entericaserotype Typhimurium
10:45 AM	107	GASTROENTERIC DISEASES	Development of a modified live vaccine against enterotoxigenicEscherichia coli-associated porcine post-weaning diarrhea
10:45 AM	146	RESPIRATORY DISEASES	Inactivation of Swine Influenza Virus with imidazolidinyl urea with retention of hemagglutination activity

Time	Oral #	Section	Monday By-The-Day Title
10:45 AM	172	VECTOR-BORNE AND PARASITIC DISEASES	The ecology of eastern equine encephalitis virus in wildlife and mosquitoes in Minnesota
10:45 AM	184	VIRAL PATHOGENESIS	Comparison of Asian highly-pathogenic PRRSV isolates to US isolates for their ability to cause secondary bacterial infection in swine
11:00 AM	51	EPIDEMIOLOGY AND ANIMAL HEALTH ECONOMICS	Post-vaccination monitoring and surveillance for Highly Pathogenic Avian Influenza in Long An Province, Vietnam, 2009: design and findings
11:00 AM	82	FOOD AND ENVIRONMENTAL SAFETY	Multi-level analysis ofCampylobacter flock status at post-chill and risk factors within the grow-out environment
11:00 AM	108	GASTROENTERIC DISEASES	Glucose significantly affects enterotoxigenic Escherichia coli adherence to intestinal epithelial cells through its effects on heat-labile enterotoxin production
11:00 AM	122	IMMUNOLOGY	Nanoparticle based inactivated adjuvanted porcine reproductive and respiratory syndrome virus vaccine elicits superior cross protective immunity
11:00 AM	147	RESPIRATORY DISEASES	Full genome of swine influenza (H1N1) in pigs using next generation sequencing
11:00 AM	173	VECTOR-BORNE AND PARASITIC DISEASES	Anthelmintic effect of proanthocyanidin extract of cranberry leaf powder onHaemonchus contortus andCaenorhabiditis elegans
11:00 AM	185	VIRAL PATHOGENESIS	Changes in circulating and thymic lymphocyte populations following infection with strains of North American or Highly Pathogenic PRRSV.
11:15 AM	52	EPIDEMIOLOGY AND ANIMAL HEALTH ECONOMICS	Evaluation of molecular profiling tools to differentiate strains of Salmonella Enteriditis
11:15 AM	83	FOOD AND ENVIRONMENTAL SAFETY	Serotype distribution and antimicrobial resistance profiles of Salmonella, E. coli, and Campylobacter isolates obtained from three broiler production systems in Ontario
11:15 AM	109	GASTROENTERIC DISEASES	Laser capture microdissection coupled with RNA-seq analysis to evaluate the transcriptional response of pigs experimentally infected with Lawsonia intracellularis
11:15 AM	123	IMMUNOLOGY	H9e peptide hydrogel: a novel adjuvant for PRRS modified live virus vaccine
11:15 AM	148	RESPIRATORY DISEASES	Genome evolution and antigenic variation of canine influenza virus H3N8 in U.S. dogs
11:15 AM	174	VECTOR-BORNE AND PARASITIC DISEASES	The chlamydiosis pathogenesis studies at experimental infection of white rats
11:15 AM	186	VIRAL PATHOGENESIS	Swine tracheobronchial lymph node mRNA responses in swine infected with a highly pathogenic strain of Porcine Reproductive and Respiratory Syndrome virus.

Time	Oral #	Section	Monday By-The-Day Title
1:15 PM	11	BACTERIAL PATHOGENESIS	Evaluation of bovine neutrophil activation byLeptospira
1:30 PM	12	BACTERIAL PATHOGENESIS	Lymphocyte subpopulations influence murine susceptibility to the agent of epizootic bovine abortion.
1:30 PM	23	BIOSAFETY AND BIOSECURITY	Carriage probability of avian influenza viruses in wild waterfowl influenced by host and environmental factors
1:30 PM	53	EPIDEMIOLOGY AND ANIMAL HEALTH ECONOMICS	Comparison of PCR assays for reliable, early and fast detection of PRRSV in different sample types from experimentally infected boars
1:30 PM	84	FOOD AND ENVIRONMENTAL SAFETY	Prevalence and fluorquinolone-susceptibilities of Campylobacter and Salmonella in cattle feces from feedlots that use fluoroquinolone therapy for bovine respiratory disease
1:30 PM	110	GASTROENTERIC DISEASES	Development of novel vaccines for mitigation of Campylobacter in poultry
1:30 PM	124	IMMUNOLOGY	Distinguished Veterinary Immunologist: Moving Swine Immunology Forward Through Molecular and Vaccine Technology
1:30 PM	187	VIRAL PATHOGENESIS	Attenuation of porcine reproductive and respiratory syndrome virus by molecular breeding of the virus envelope genes from genetically divergent strains
1:45 PM	13	BACTERIAL PATHOGENESIS	Challenge study to assess association between Moraxella bovoculi and Infectious bovine Keratoconjunctivitis in calves
1:45 PM	24	BIOSAFETY AND BIOSECURITY	Electronic microarrays for detection and typing of high consequence agents in swine
1:45 PM	54	EPIDEMIOLOGY AND ANIMAL HEALTH ECONOMICS	Swine influenza virus dynamics in sow herds over time
1:45 PM	85	FOOD AND ENVIRONMENTAL SAFETY	Temporal changes in antimicrobial resistance within Michigan dairy cows
1:45 PM	111	GASTROENTERIC DISEASES	Comparative pathogenicity of porcine rotavirus group A, B and C in neonatal pigs
1:45 PM	149	RESPIRATORY DISEASES	Zoonotic tuberculosis in pastoralists and their livestock in Ethiopia
1:45 PM	188	VIRAL PATHOGENESIS	Development of a modified live vaccine against porcine reproductive and respiratory syndrome with optimal “DIVA” marker potential
2:00 PM	14	BACTERIAL PATHOGENESIS	Comparison of induced small animal models for Guillain Barre syndrome (GBS) as post infectious sequelae toCampylobacter jejuni infection
2:00 PM	25	BIOSAFETY AND BIOSECURITY	Serotype reactivity of commercial immunoassays forSalmonella enterica identification in experimentally-inoculated equine fecal samples
		(continued)	

Time	Oral #	Section	Monday By-The-Day Title
2:00 PM	55	EPIDEMIOLOGY AND ANIMAL HEALTH ECONOMICS	Antimicrobial susceptibilities ofEscherichia coli isolated from feces of swine fed with chlortetracycline or copper
2:00 PM	86	FOOD AND ENVIRONMENTAL SAFETY	Prevalence of pathogenic shiga toxin producing Escherichia coli in dairy cattle and wildlife in Texas
2:00 PM	112	GASTROENTERIC DISEASES	Characterization of porcine group B rotavirus G genotype in the United States reveals substantial genetic diversity
2:00 PM	150	RESPIRATORY DISEASES	The impact of gastrointestinal nematode parasitism on the response of calves to viral respiratory vaccination
2:00 PM	189	VIRAL PATHOGENESIS	Flexible polymer adjuvants for live and inactivated vaccines: Application to PRRS live vaccine
2:15 PM	15	BACTERIAL PATHOGENESIS	Cellulutis in turkeys and the role of gut integrity
2:15 PM	26	BIOSAFETY AND BIOSECURITY	Environmental survival of Equid Herpesvirus -1.
2:15 PM	56	EPIDEMIOLOGY AND ANIMAL HEALTH ECONOMICS	Role of environment in the persistence of antimicrobial resistant Salmonella in antimicrobial free (ABF) and conventional pigs at farm and slaughter
2:15 PM	87	FOOD AND ENVIRONMENTAL SAFETY	Epidemiology of Shiga toxin-producing Escherichia coli (STEC) shedding in finishing swine- a descriptive longitudinal study
2:15 PM	113	GASTROENTERIC DISEASES	Characterization of swine group C rotavirus G genotypes from the United States and Canada reveals a new proposed G genotype
2:15 PM	125	IMMUNOLOGY	Development of a mouse model for delineating protective immune response(s) specific for epizootic bovine abortion
2:15 PM	190	VIRAL PATHOGENESIS	Novel simian hemorrhagic fever viruses from wild African primates offer new insights into the evolutionary origins of PRRSV
2:30 PM	16	BACTERIAL PATHOGENESIS	Optimization ofin vitrogrowth conditions and DNA extraction from Treponema phagedenis isolated from bovine digital dermatitis lesions.
2:30 PM	27	BIOSAFETY AND BIOSECURITY	Efficacy of Sodium Dodecyl Sulfate and Formic acid inactivation of Caprine Arthritis-Encephalitis virus in vitro
2:30 PM	57	EPIDEMIOLOGY AND ANIMAL HEALTH ECONOMICS	Risk factors for environmental contamination withSalmonellaenterica in a veterinary teaching hospital
2:30 PM	88	FOOD AND ENVIRONMENTAL SAFETY	Escherichia coli O104 is prevalent in feces of feedlot cattle, but isolated strains did not carry genes characteristic of enterohemorrhagic or enteroaggregative pathotype
2:30 PM	114	GASTROENTERIC DISEASES	The effect of climate change on the evolution of food- and waterborne diseases: a systematic review.

Time	Oral #	Section	Monday By-The-Day Title
2:30 PM	126	IMMUNOLOGY	Use of dermal fibroblasts to predict the innate immune response to bovine mastitis
2:30 PM	152	RESPIRATORY DISEASES	In vitro and in vivo prostaglandin E2 synthesis in BRSV infection and modulation by COX inhibition
2:30 PM	191	VIRAL PATHOGENESIS	Validation of an equine arteritis virus antibody cELISA according to OIE protocol.
3:00 PM	17	BACTERIAL PATHOGENESIS	Use of anti-SUAM antibodies in a Passive protection model to prevent Streptococcus uberis mastitis
3:00 PM	28	BIOSAFETY AND BIOSECURITY	Biosafety and Biosecurity Section Keynote: African Swine Fever: Current Situation and Control Strategy
3:00 PM	58	EPIDEMIOLOGY AND ANIMAL HEALTH ECONOMICS	Perceptions of veterinarians and producers concerning Johne's disease in US beef cow-calf operations
3:00 PM	89	FOOD AND ENVIRONMENTAL SAFETY	Antibiotic use versus antibiotic resistance profiles of commensalE.coli in beef cattle: explaining their association via bacterial growth parameters
3:00 PM	127	IMMUNOLOGY	The potential contribution of epigenetic modifications to the animal-specific responses of dermal fibroblasts to LPS.
3:00 PM	153	RESPIRATORY DISEASES	Prevalence of viral and bacterial pathogens in nasopharyngeal and pharyngeal recess regions of Holstein calves with and without signs of clinical bovine respiratory disease
3:00 PM	192	VIRAL PATHOGENESIS	Isolation of a novel swine influenza virus distantly related to influenza C
3:15 PM	18	BACTERIAL PATHOGENESIS	Defining the role of SUAM in the pathogenesis of Streptococcus uberis mastitis using a SUAM-negative gene deletion mutant
3:15 PM	59	EPIDEMIOLOGY AND ANIMAL HEALTH ECONOMICS	Effect of individual animal calving pens on peripartum transmission of Mycobacterium avium subsp. paratuberculosis in Holstein heifer calves.
3:15 PM	90	FOOD AND ENVIRONMENTAL SAFETY	Commercial evaluation of an SPR-containingEscherichia coli bacterial extract vaccine
3:15 PM	128	IMMUNOLOGY	Interleukin-8 receptor expression in bovine mammary tissue.
3:15 PM	154	RESPIRATORY DISEASES	Pathogenicity ofBibersteinia trehalosi in cattle
3:15 PM	193	VIRAL PATHOGENESIS	Harnessing RNAi to inhibit avian influenza replication in avian cells using a novel delivery technology: Progressing towards an alternative prevention strategy.
3:30 PM	19	BACTERIAL PATHOGENESIS	Transcriptome expression profiles of Streptococcus uberis during bovine mastitis
3:30 PM	60	EPIDEMIOLOGY AND ANIMAL HEALTH ECONOMICS	Effect of delayed exposure of dairy cattle to Mycobacterium avium subsp. paratuberculosis on age at first test positive and clinical Johne's disease
		(continued)	

Time	Oral #	Section	Monday By-The-Day Title
3:30 PM	91	FOOD AND ENVIRONMENTAL SAFETY	Evaluation of plasmid stability in green fluorescent protein-labeled Escherichia coli O157 in a non-selective, nutrient deficient environment
3:30 PM	129	IMMUNOLOGY	Lipid metabolism by bovine mammary endothelial cells duringStreptococcus uberis mastitis
3:30 PM	155	RESPIRATORY DISEASES	RNA-Seq based structural re-annotation of BRD bacterial pathogens
3:30 PM	194	VIRAL PATHOGENESIS	Pathogenicity and transmissibility of novel reassortant H3N2 swine influenza viruses with the 2009 pandemic H1N1 genes in pigs
3:45 PM	20	BACTERIAL PATHOGENESIS	Next-generation sequencing of Streptococcus uberis UT888 genome facilitates quest for virulence /pathogenic associated gene features
3:45 PM	29	BIOSAFETY AND BIOSECURITY	Complying with U.S. export controls as a life science researcher
3:45 PM	61	EPIDEMIOLOGY AND ANIMAL HEALTH ECONOMICS	Does colostrum intake affect the development of the rectal microbiota in pre-weaning dairy calves?
3:45 PM	92	FOOD AND ENVIRONMENTAL SAFETY	Effect of flavophospholipol and environment on antimicrobial resistance in beef cattle.
3:45 PM	130	IMMUNOLOGY	Increased linoleic acid in post-partum bovine monocytes is associated with proinflammatory phenotype.
3:45 PM	156	RESPIRATORY DISEASES	Respiratory Diseases Section Keynote: Mannheimia haemolytica immunity. Are we there yet?
3:45 PM	195	VIRAL PATHOGENESIS	Development of an equine ocular endothelial cell model to study equine herpesvirus myelitis (EHM)
4:00 PM	21	BACTERIAL PATHOGENESIS	Mechanisms of intrinsic resistance to antimicrobial peptides of Edwardsiella ictaluri and its influence on fish gut inflammation and virulence.
4:00 PM	30	BIOSAFETY AND BIOSECURITY	Development and implementation of an HSEEP compliant avian influenza response training exercise for zoological personnel.
4:00 PM	62	EPIDEMIOLOGY AND ANIMAL HEALTH ECONOMICS	Metagenomic versus microbiological culture based approaches to evaluate the effects of interventions strategies on ceftiofur and tetracycline resistance in cattle feces
4:00 PM	93	FOOD AND ENVIRONMENTAL SAFETY	Discovery of novel alternatives to antibiotic growth promoter to protect food safety
4:00 PM	131	IMMUNOLOGY	Age-related susceptibility to R equi infection in foals
4:15 PM	22	BACTERIAL PATHOGENESIS	Penicillin-binding proteins and cefoxitin in Staphylococcus pseudintermedius and Staphylococcus schleiferi subspecies coagulans
4:15 PM	63	EPIDEMIOLOGY AND ANIMAL HEALTH ECONOMICS	Asymptomatic endemicChlamydia pecorum infections reduce growth rates in calves by up to 48 percent
4:15 PM	94	FOOD AND ENVIRONMENTAL SAFETY	Prevalence of transferable copper resistance gene,tcrB, in fecal enterococci of feedlot cattle fed diets supplemented with copper

Time	Oral #	Section	Tuesday By-The-Day Title
8:00 AM	31	COMPANION ANIMAL EPIDEMIOLOGY	Companion Animal Epidemiology Section Keynote to be presented in Salon A/B/C/D Room, 5th Floor: From licking stamps to clicking buttons - moving from conventional questionnaires to online surveys
8:15 AM	132	IMMUNOLOGY	Evaluation of a live attentuated vaccine for Johne's disease
8:15 AM	157	RESPIRATORY DISEASES	DNA shuffling of the GP3 genes of PRRSV produces a chimeric virus with an improved cross-neutralizing ability against a heterologous PRRSV strain
8:30 AM	133	IMMUNOLOGY	Tumor necrosis factor (TNF)-α diminishes the ability of bovine macrophage to cleave extracellular traps formed in response to M. haemolytica
8:30 AM	158	RESPIRATORY DISEASES	Characterization of the neutralizing antibody response to PRRSV
8:45 AM	32	EPIDEMIOLOGY AND ANIMAL HEALTH ECONOMICS	Mark Gearhart Memorial Graduate Student Award: Nasal shedding of Equine Herpesvirus-1 from horses in an outbreak of Equine Herpes Myeloencephalopathy in Western Canada
8:45 AM	134	IMMUNOLOGY	Induction of osteopontin expression in bovine mammary endothelial cells
8:45 AM	159	RESPIRATORY DISEASES	Development of swine oral fluid based porcine reproductive and respiratory syndrome virus neutralizing assay: a potential diagnostic tool for PRRS herd immunity
8:45 AM	196	VIRAL PATHOGENESIS	Group C porcine Rotavirus subunit vaccine
9:00 AM	33	COMPANION ANIMAL EPIDEMIOLOGY	Risk factors for antimicrobial resistant Salmonella spp. and Escherichia coli carriage in pet dogs from volunteer households in Ontario (2005-2006)
9:00 AM	64	EPIDEMIOLOGY AND ANIMAL HEALTH ECONOMICS	Livestock Deaths in Mangarabombang Subdistrict, Takalar District, South Sulawesi Province, Indonesia, 2011-2012: Application of Epidemiological Investigation
9:00 AM	95	FOOD AND ENVIRONMENTAL SAFETY	An agent-based model to assess the potential effects of vaccines in Escherichia coli O157 shedding and transmission in feedlots
9:00 AM	135	IMMUNOLOGY	Genetic variation in CXCR1 amino acid expression significantly tied to clearance of Streptococcus uberis in an intramammary challenge model
9:00 AM	160	RESPIRATORY DISEASES	Effect of sample collection material on the detection of PRRSV in oral fluid
9:00 AM	197	VIRAL PATHOGENESIS	Genetic diversity of porcine circoviruses type 2 detected in pigs in Ukraine
9:15 AM	34	COMPANION ANIMAL EPIDEMIOLOGY	Syndromic surveillance for nosocomial infections in small animal veterinary referral hospitals
		(continued)	

Time	Oral #	Section	Tuesday By-The-Day Title
9:15 AM	96	FOOD AND ENVIRONMENTAL SAFETY	The impact of vaccination and post-harvest intervention failures on beef carcass contamination with E. coli O157
9:15 AM	161	RESPIRATORY DISEASES	Probability of detecting PRRSV infection using pen-based swine oral fluid specimens as a function of within-pen prevalence
9:15 AM	198	VIRAL PATHOGENESIS	Characterization of the first complete genome sequence of the North American beaver (Castor canadensis) papillomavirus
10:00 AM	35	COMPANION ANIMAL EPIDEMIOLOGY	Survey to investigate pet ownership and attitudes to pet care in metropolitan Chicago dog and/or cat owners.
10:00 AM	66	EPIDEMIOLOGY AND ANIMAL HEALTH ECONOMICS	Using quarterly earnings to assess return to function in Thoroughbred racehorses after either modified laryngoplasty or colic surgery
10:00 AM	97	FOOD AND ENVIRONMENTAL SAFETY	Development of a loop-mediated isothermal amplification assay for point-of-need detection of Escherichia coli
10:00 AM	162	RESPIRATORY DISEASES	Detection of PRRSV antibody in oral fluid specimens from individual boars using a commercial prrsv serum antibody elisa.
10:00 AM	199	VIRAL PATHOGENESIS	Expression of type I interferon-induced antiviral state during experimental infection with low or high virulence bovine viral diarrhea virus in beef calves
10:15 AM	36	COMPANION ANIMAL EPIDEMIOLOGY	Birth and death rate estimates and selected owner demographic data associated with cat, dog, pet bird, and horse ownership in U.S. households in 2006
10:15 AM	67	EPIDEMIOLOGY AND ANIMAL HEALTH ECONOMICS	Minimization of bovine tuberculosis control costs in US cattle herds
10:15 AM	98	FOOD AND ENVIRONMENTAL SAFETY	Evaluating on-farm interventions to reduce antimicrobial resistance in enteric commensal Escherichia coli of cattle with mathematical modeling
10:15 AM	163	RESPIRATORY DISEASES	Ring test evaluation for the detection of PRRSV antibody in oral fluid specimens using a commercial PRRSV serum antibody ELISA.
10:15 AM	200	VIRAL PATHOGENESIS	Differential expression of pro-inflammatory and anti-inflammatory cytokines during experimental infection with low or high virulence bovine viral diarrhea virus in beef calves
10:30 AM	37	COMPANION ANIMAL EPIDEMIOLOGY	Use of survival analysis to assess the effects of fee structure on post-adoption relinquishment of dogs and cats
10:30 AM	68	EPIDEMIOLOGY AND ANIMAL HEALTH ECONOMICS	Patterns of cattle farm visitation by white-tailed deer in relation to bovine tuberculosis transmission risk in Minnesota

Time	Oral #	Section	Tuesday By-The-Day Title
10:30 AM	99	FOOD AND ENVIRONMENTAL SAFETY	Impact of feeding distillers grain-based diets on the colonic microbial community structure of cattle
10:30 AM	164	RESPIRATORY DISEASES	The antiviral activity of Actinobacillus pleuropneumoniae against Porcine reproductive and respiratory syndrome virus in the porcine alveolar macrophages
10:30 AM	201	VIRAL PATHOGENESIS	PCR-screening of chlamydia and viral contamination of bovine semen in Ukraine
10:45 AM	38	COMPANION ANIMAL EPIDEMIOLOGY	Risk factors for the development of malignant histiocytosis in Bernese Mountain Dogs
10:45 AM	69	EPIDEMIOLOGY AND ANIMAL HEALTH ECONOMICS	A comparison of real and synthetic population datasets for simulation modeling of highly pathogenic avian influenza (H5N1) in commercial poultry flocks in South Carolina.
10:45 AM	100	FOOD AND ENVIRONMENTAL SAFETY	An analysis of foodborne illness risk factor violations and bacterial load in restaurant food preparation areas.
10:45 AM	202	VIRAL PATHOGENESIS	Viral Pathogenesis Section Keynote: Of Men, Pigs, Birds and ...Flu.
11:00 AM	39	COMPANION ANIMAL EPIDEMIOLOGY	Prevalence of feline influenza virus infection in cats in Bangladesh.
11:00 AM	70	EPIDEMIOLOGY AND ANIMAL HEALTH ECONOMICS	Density and distribution of backyard poultry flocks in metropolitan Denver, Colorado
11:00 AM	101	FOOD AND ENVIRONMENTAL SAFETY	In vitro effect of deoxynivalenol (DON) mycotoxin on porcine circovirus type 2 (PCV2) replication and cytopathic effect.
11:15 AM	40	COMPANION ANIMAL EPIDEMIOLOGY	The reliability of a survey to score cat socialization from unsocialized to highly socialized
11:15 AM	71	EPIDEMIOLOGY AND ANIMAL HEALTH ECONOMICS	Antimicrobial resistance in fecal E.coli of Holstein calves housed individually or in group pens.
11:15 AM	102	FOOD AND ENVIRONMENTAL SAFETY	A one health approach to public health issues in Ghana

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	Methicillin-resistant *Staphylococcus aureus* and *Staphylococcus pseudintermedius* from companion animals and horses at a veterinary teaching hospital in Quebec, Canada.	**E. Rodriguez**[1], S. Messier[1], D. Daignault[2], S. Monecke[3], R. Ehricht[4], M. Archambault[1]; [1]Department of Pathology and Microbiology, Faculty of Veterinary Medicine, University of Montreal, Saint Hyacinthe, QC, Canada, [2]Canadian Integrated Program for Antimicrobial Resistance Surveillance, Health Canada, Saint Hyacinthe, QC, Canada, [3]Institute for Medical Microbiology and Hygiene, Technical University of Dresden, Dresden, Germany, [4]Alere Technologies GmbH, Jena, Germany.
002P	A new drug for an old bug: Antimicrobial activity of novel substituted thiazoles against methicillin-resistant Staphylococcus aureus (MRSA)	**H. Mohammad**[1], A.S. Mayhoub[2], A. Ghafoor[1], M. Soofi[1], R.A. Alajlouni[1], M. Cushman[2], M.N. Seleem[1]; [1]Comparative Pathobiology, Purdue University, West Lafayette, IN, USA, [2]Medicinal Chemistry and Molecular Pharmacology, Purdue University, West Lafayette, IN, USA
003P	Characterization of the ability of coagulase negative staphylococci isolated from milk to form biofilms.	Y.D.N. Tremblay[1], D. Lamarche[1], P. Chever[1], D. Haine[2], S. Messier[1], **M. Jacques**[1]; [1]Pathologie et Microbiologie, Université de Montréal, St-Hyacinthe, QC, Canada, [2]Sciences cliniques, Université de Montréal, St-Hyacinthe, QC, Canada.
004P	Cj0843c, a putative lytic transglycosylase, is involved in beta-lactam resistance by modulating beta-lactamse activity in Campylobacter jejuni	**X. Zeng**, S. Brown, B. Gillespie, J. Lin; Animal Science, University of Tennessee, Knoxville, TN, USA.
005P	Inactivation of *gidB* confers low-level streptomycin resistance and compromises bacterial fitness in *Campylobacte* r	**Z. Shen**, L. Dai, Z. Wu, Q. Zhang; Iowa State University, Ames, IA, USA.
006P	Salmochelin-mediated iron acquisition in Campylobacter jejuni	**Y. Mo**, X. Zeng, J. Lin; Department of Animal Science, The University of Tennessee, Knoxville, TN, USA.
007P	Research in progress: A bivalent immunocontraceptive vaccine against brucellosis in feral swine	**G.P. Smith**[1], P. Rajasekaran[1], S.M. Boyle[1], L.A. Miller[2], N. Sriranganathan[1]; [1]VA-MD Regional College of Veterinary Medicine, Blacksburg, VA, USA, [2]USDA National Wildlife Research Center, Fort Collins, CO, USA
008P	Immunogenicity and safety of a natural rough mutant of *Brucella suis* as a vaccine for swine	**S. Olsen**[1], C.A. Johnson[1], W. Stoffregen[2]; [1]National Animal Disease Center, Ames, IA, USA, [2]Preclinical Pathology, Boston Scientific Corporation, Plymouth, MN, USA
009P	Isolation of *Brucella* species from aborted fetuses of sheep and goats in Mongolia	**K. Lee**[1], M. Her[1], J.-Y. Kim[1], S.-I. Kang[1], E. Janchivdorj[2], S. Jung[1]; [1]Bacterial disease division, Animal, Plant and Fisheries Quarantine and Inspection Agency, Anyang, Korea, Republic of, [2]Immunological Research Center, Institute of Veterinary Medicine, Ulaanbaatar, Mongolia.

BACTERIAL PATHOGENESIS POSTERS
Poster Session I - Sunday 6:30-8:00 PM - Grand Ballroom Salon III - 7th floor
Section Leader: Gireesh Rajashekara

No.	Title	Authors
010P	Colonization kinetics of a lipoprotein 28 deficient mutant of *B. abortus* S19	**A.M. Dougherty**[1], G. Vernati[1], J. Leonhardt[1], J.E. Lowry[2], G. Andrews[1]; [1]Veterinary Sciences, University of Wyoming, Laramie, WY, USA, [2]Department of Clinical Investigations, Eisenhowser Army Medical Center, Fort Gordon, GA, USA.
011P	Motility of Filamentous Cells of Salmonella enterica	**J. Tsarouha**[1], N. Faith[1], C. Kaspar[2], A. Wong[2], C.J. Czuprynski[1]; [1]University of Wisconsin - Madison, Madison, WI, USA, [2]University of Wisconsin, Madison, WI, USA
012P	Identification of immunogenic *Brucella canis* outer membrane proteins.	A. Heredia-Antúnez[1], G. Palomares-Resendiz[2], E. Díaz-Aparicio[2], F. Suárez-Güemes[1], **B. Arellano-Reynoso**[1]; [1]Faculty of Veterinary Medicine,, National University of Mexico, Mexico, D.F, Mexico, [2]CENID-Microbiología, INIFAP, Mexico, D.F, Mexico
013P	Survival and virulence of Salmonella spp. in poultry feed	**A. Andino**, S. Pendleton, N. Zhang, I. Hanning; Food Science and Technology, University of Tennessee, Knoxville, TN, USA.
014P	Analysis of the cecal microbial profiles of commercial layer chickens with *Escherichia coli*-induced peritonitis.	**E.M.K. Kurundu Hewage**[1], D.S. Wijetunge[1], P. Gunawardana[2], S. Kariyawasam[1]; [1]Veterinary and Biomedical Sciences, Pennsylvania State University, University Park, PA, USA, [2]Hillandale Farms, Gettysburg, PA, USA
015P	Bacterial community profiling of tonsils from diseased pigs using terminal restriction fragment length polymorphism analysis	**S. Kernaghan**[1], D. Slavic[2], S. Chen[2], Z. Poljak[3], J.I. MacInnes[1]; [1]Department of Pathobiology, University of Guelph, Guelph, ON, Canada, [2]Animal Laboratory Services, University of Guelph, Guelph, ON, Canada, [3]Department of Population Medicine, University of Guelph, Guelph, ON, Canada.
016P	Expression of adhesin genes of *Actinobacillus suis* grown under conditions that mimic the host environment	**A.R. Bujold**, J.I. MacInnes; Department of Pathobiology, University of Guelph, Guelph, ON, Canada.
017P	Molecular characterization of a surface protein endowed with endonuclease activity related to the restriction enzymes of the RE_*AlwI* superfamily in *Mycoplasma meleagridis*	**B. Ben Abdelmoumen Mardassi, Sr.**, E. Yacoub, Jr, A. Bejaoui Khiari, Jr, N. Hechmi, jr, B. Mlik, Sr; Molecular Microbiology, Vaccinology and Biotechnology Development, Institut Pasteur de Tunis, Tunis, Tunisia.
018P	A fatal case of Arcanobacterium pyogenes in 9-year-old Korean native cattle	**Y.H. Kim**, K.H. Lee, S.S. Yoon, W.H. Park, M.Y. Rhyoo, M.H. Lee; Animal, Plant and Fisheries Quarantine and inspection Agency (QIA), Anyang, Korea, Republic of.
019P	Prevalence of Coxiella burnetii in a healthy bighorn sheep population	J. Ninneman, W. Stensland, **R. Dewell**, P. Wolff, E. Strait, P. Plummer; VDPAM, Iowa state university, Ames, IA, USA.
020P	Zebrafish larvae as model to evaluate lipopolysaccharide toxicity	**A. Loh**, T. Martin, R. Curtiss, J. Santander; Arizona State University, Tempe, AZ, USA.

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BACTERIAL PATHOGENESIS POSTERS
Poster Session I - Sunday 6:30-8:00 PM - Grand Ballroom Salon III - 7th floor
Section Leader: Gireesh Rajashekara

No.	Title	Authors
021P	Application of Raman spectroscopy in antimicrobial drug discovery research	**R.A. Alajlouni**[1], A.I.M. Athamneh[2], A.S. Mayhoub[3], M. Cushman[3], R.S. Senger[2], M.N. Seleem[1]; [1]Comparative Pathobiology, Purdue College of Veterinary Medicine, Purdue University, West Lafayette, IN, USA, [2]Biological Systems Engineering, Virginia Tech, Blacksburg, VA, USA, [3]Medicinal Chemistry and Molecular Pharmacology, purdue university, west lafayette, IN, USA.
022P	New drugs for bad bugs: class II HMG-CoA reductase inhibitors	**D. McPherson**[1], D. López-Pérez[2], C.N. Steussy[3], M. Lipton[2], C.V. Stauffacher[3], M.N. Seleem[1]; [1]Department of Comparative Pathobiology, Purdue College of Veterinary Medicine, West Lafayette, IN, USA, [2]Department of Chemistry, Purdue University, West Lafayette, IN, USA, [3]Department of Biological Sciences, Purdue University, West Lafayette, IN, USA
023P	Use of matrix-assisted laser desorption/ionization time-of-flight mass spectrometry to detect *Streptococcus suis* variants	**T. Frana**, L. McDeid, D. Adams, C. Thompson; Iowa State University, Ames, IA, USA.
024P	Detection and identification of a new species of *Mycoplasma* in swine	**I. Mandeville**[1], C. Girard[2], D. Tremblay[1], V. Allard[1], M. Denicourt[3], J. Harel[4], C. Gagnon[4]; [1]Service diagnostic, Faculté de médecine vétérinaire, Université de Montréal, Saint-Hyacinthe, QC, Canada, [2]Département de pathologie et microbiologie, Faculté de médecine vétérinaire, Université de Montréal, Saint-Hyacinthe, QC, Canada, [3]Département de sciences cliniques, Faculté de médecine vétérinaire, Université de Montréal, Saint-Hyacinthe, QC, Canada, [4]Centre de recherche en infectiologie porcine (CRIP), Faculté de médecine vétérinaire, Université de Montréal, Saint-Hyacinthe, QC, Canada.
025P	Evaluation of diagnostic tests for the assessment of human brucellosis in Georgia	N. Chitadze[1], L. Malania[1], L. Sanodze[1], N. Garuchava[1], M. Ramishvili[1], M. Grdzelidze[1], T. Akhvlediani[2], N. Kokaia[3], M. Broladze[1], S. Chubinidze[1], S. Tsanava[1], M. Nikolich[4], R. Rivard[5], P. Elzer[6], **N. Trapaidze**[2]; [1]National Center for Disease Control and Public Health, Tbilisi, Georgia, [2]WRAIR/USAMRIID Clinical Research Unit, Tbilisi, Georgia, [3]Medical Parasitology and Tropical Medicine Research Institute, Tbilisi, Georgia, [4]Walter Reed Army Institute of Research, Silver Spring, MD, USA, [5]U.S. Army Medical Research Institute of Infectious Diseases, Fort Detrick, MD, USA, [6]School of Animal Sciences, Louisiana State University AgCenter, Baton Rouge, LA, USA.

BACTERIAL PATHOGENESIS POSTERS
Poster Session I - Sunday 6:30-8:00 PM - Grand Ballroom Salon III - 7th floor
Section Leader: Gireesh Rajashekara

No.	Title	Authors
026P	Investigation on the diagnostic efficiency of STAT for brucellosis	**S.-R. Sung**, J.-Y. Kim, M. Her, K. Lee, J. Gu, S.-I. Kang, H. Lee, S. Jung; Bacterial disease division, Animal, plant and fisheries Quarantine and Inspection Agency, Anyang, Korea, Republic of.

BIOSAFETY AND BIOSECURITY POSTERS
Poster Session I - Sunday 6:30-8:00 PM - Grand Ballroom Salon III - 7th floor
Section Leader: Gabriele Landolt
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
027P	Experience of implementing international recommendations for control recombinant DNA safety in Ukraine	**O.S. Solodiankin**, A.P. Gerilovych, V.I. Bolotin, I.V. Goraichuk; National Scientific Center Institute of Experimental and Clinical Veterinary Medicine, Kharkiv, Ukraine.
028P	Study of the effect of metallic nanoparticles on the growth properties of discrete and associated forms of *Pasteurella multocida*	**K. Olena, III**; Swine Disease Research, National Scientific Centre, Kharkiv, Ukraine.

COMPANION ANIMAL EPIDEMIOLOGY POSTERS
Poster Session I - Sunday 6:30-8:00 PM - Grand Ballroom Salon III - 7th floor
Section Leaders: Margaret Slater 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
029P	Veterinary Epidemiology of Rabies in Ukraine	**S. Nychyk**, I. Polupan; Institute of Veterinary Medicine, Kyiv, Ukraine.
030P	*Escherichia coli* with CTX-M-15 type ESBL isolated from urinary samples of dogs and cats	**H. Huber**[1], C. Zweifel[2], S. Prohaska[1], E. Huebschke[1], M.M. Wittenbrink[1], R. Stephan[2]; [1]Institute of Veterinary Bacteriology Vetsuisse Faculty, University of Zurich, Zurich, Switzerland, [2]Institute for Food Safety and Hygiene, Vetsuisse Faculty, University of Zurich, Zurich, Switzerland.
031P	Detection of Mycoplasma gallisepticum natural reservoirs among waterfowl	**O. Obukhovska, Sr.**; Department of Bacterial Infection, National Scientific Center, Institute of Experimental and Clinical Medicine, Kharkiv, Ukraine.

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
032P	Efficacy of a lacteal-derived colostrum replacer feeding program for preventing failure of passive transfer in calves.	**P. Pithua**[1], S.S. Aly[2], D.H. Haines[3], J. Champagne[2], J.R. Middleton[1], S.E. Poock[1]; [1]Department of Veterinary Medicine and Surgery, University of Missouri, Columbia, MO, USA, [2]Population Health and Reproduction, University of California, Davis, CA, USA, [3]Veterinary Microbiology, University of Saskatchewan, Saskatoon, SK, Canada
033P	Transmission of *Brucella abortus* in calves younger than 3 months diagnosed using the card and the immunodiffusion tests in two dairy herds in the state of Queretaro.	I. Carrizosa[1], M. Medina[1], G.E. Palomares[2], **E. Diaz**[2]; [1]Centro de Enseñanza Investigación y Extensión en Producción Animal en Altiplano, FMVZ UNAM, Mexico, Mexico, [2]Bacteriology, INIFAP, Mexico, Mexico
034P	Real time PCR detection of hemotropic *Mycoplasma* species in symptomatic dairy cattle from the Midwest United States	**A. Kreuder**[1], P. Plummer[2], A. Herrick[3], U. Donnett[3], J. Trujillo[3]; [1]VDPAM, Iowa State University, Ames, IA, USA, [2]VDPAM, VMPM, Iowa State University, Ames, IA, USA, [3]VMPM, Iowa State University, Ames, IA, USA
035P	Minimum inhibitory concentrations and antimicrobial resistance patterns of ovine and caprine field strains of *Corynebacterium pseudotuberculosis*	**K.A. Clothier**; California Animal Health & Food Safety Lab, University of California, Davis, Davis, CA, USA.
036P	Q-fever: epizootic situation and laboratory diagnostics	**L. Marushchak**, O. Nevolko, O. Volosianko, Z. Drozhzhe; SSRILDVSE, Kyiv, Ukraine.
037P	Genetic characterization and phylogenetic analysis of porcine circovirus type 2 field strains isolated from porcine circovirus disease (PCVD) pigs in Korea	**L. Munik**, S. Kim, S. Kim, C. Yoon, J. Han; kangon national university, chun-cheon, Korea, Republic of.
038P	Immunostimulatory boosting effect of anionic alkali mineral complex solution(Barodon®) on FMDVvaccine in pigs	**S.J. Kim**[1], B.W. Yoo[2], S.I. Choi[3], S.Y. Hwang[4], J.H. Han[1]; [1]College of Veterinary Medicine and Institute of Veterinary Science, Kangwon national university, Chuncheon, Korea, Republic of, [2]Cargill agri purina, Sungnam, Korea, Republic of, [3]Barodon-SF, Ansung, Korea, Republic of, [4]Microbiology Lab., Seoul national university, Seoul, Korea, Republic of
039P	Pathological investigation of multifocal interstitial nephritis from slaughtered pigs in Korea	**M. Lee**, S. Kim, S. Kim, C.-W. Yoon, J.-H. Han; Kangon National University, Chun-Cheon, Korea, Republic of.
040P	Longitudinal study of fecal contamination of cattle feed by starlings at dairy farms in Ohio	**G.A. Medhanie**[1], D.L. Pearl[1], J.T. Lejeune[2]; [1]Population Medicine, University of Guelph, Guelph, ON, Canada, [2]Food Animal and Health Research Program, The Ohio State University, Wooster, OH, USA

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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

No.	Title	Authors
041P	Multilocus variable-number tandem repeat analysis of *Salmonella* enteritidis strains isolated in brazil and north america over a 24-year period	F. Campioni[1], J.P. Falcao[1], M.A. Davis[2], M.I.C. Medeiros[3], **D.H. Shah**[4]; [1]Department of Clinical Analysis, University of São Paulo, Ribeirão Preto, Brazil, [2]Department of Veterinary Clinical Sciences, Washington State University, Pullman, WA, USA, [3]Adolfo Lutz Institute of Ribeirão Preto, Ribeirão Preto, Brazil, [4]Department of Veterinary Microbiology and Pathology, Washington State University, Pullman, WA, USA.
041aP	The frequency of detecting Shiga toxin-producing *Escherichia coli* O groups and virulence genes in feces of commercial feedlot cattle	**C. Cull**, D. Renter, L. Schaefer, Z. Paddock, X. Shi, J. Bai, T. Nagaraja; Deparment of Pathobiology/Medicine, Kansas State University, Manhattan, KS, USA.
042P	Impact of Johne's disease, natural infection and vaccination, on bovine tuberculosis diagnostics tests	**J. Ribeiro Lima**[1], E. Patton[2], G. Linda[3], B. Carlson[1], S.J. Wells[1]; [1]Veterinary Population Medicine, University of Minnesota, St. Paul, MN, USA, [2]Wisconsin Department of Trade, Agriculture and Consumer Protection, Madison, WI, USA, [3]Minnesota Board of Animal Health, St. Paul, MN, USA
043P	Network analysis of cattle movements in relation to bovine tuberculosis transmission risk in Minnesota	**J. Ribeiro Lima**[1], B. Thompson[2], M.E. Craft[1], S.J. Wells[1]; [1]Veterinary Population Medicine, University of Minnesota, St. Paul, MN, USA, [2]Minnesota Board of Animal Health, S. Paul, MN, USA.
044P	Epidemiological analysis of BVDV infection in cattle farms of Kharkov region, Ukraine	**A. Gerilovych**[1], S. Vilcek[2], E. Peterhans[3], I. Goraichuk[1], A. Jackova[2], V. Bolotin[1], O. Solodiankin[1]; [1]National Scientific Center Institute of Experimental and Clinical Veterinary Medicine, Kharkov, Ukraine, [2]Department of Epizootiology and Parasitology, University of Veterinary Medicine and Pharmacy, Kosice, Slovakia, [3]Institute of Veterinary Virology, University of Bern, Bern, Switzerland

FOOD AND ENVIRONMENTAL SAFETY 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
045P	Comparison of *M. bovis* gamma interferon test results between tissue culture plate and microtube methods	**A. Hill**, M. Davidson; California Animal Hlth & Food Safety Lab, University of California-Davis, Davis, CA, USA.
046P	Reaction of the Erysipelothrix rhusiopathiae species on weeds' influence	**O. Zhukorskyi**; NAAS, Kyiv, Ukraine.
047P	Molecular detection of Salmonella in environmental samples from meat processing facilities in Mexico	**A. Tudor**, K. Nightingale, M. Brashears; Texas Tech University, Lubbock, TX, USA.
048P	Development of a multiplex real-time PCR for the serotype-specific detection of *Salmonella* Enteritidis	F. Campioni, L. Orfe, R. Crespo, **D.H. Shah**; Department of Veterinary Microbiology and Pathology, Washington State University, Pullman, WA, USA.
049P	*Salmonella* shedding in close-up dairy heifers.	**A. Mergener**, L. Neuder, J. Funk; Large Animal Clinical Sciences, Michigan State University, East Lansing, MI, USA.
050P	Modeling *Salmonella* dynamics within a finishing pig farm: group structure effects on transmission	E. Crosley[1], A. Nivens[2], I. Rubin[3], **C. Lanzas**[4], S. Lenhart[5], M. Lelu[6], T. Phan[5]; [1]Mathematics, Bowdoin College, Brunswick, ME, USA, [2]Mathematics, Maryville College, Maryville, TN, USA, [3]Ecology and Evolutionary Biology, Cornell University, Ithaca, NY, USA, [4]Biomedical and Diagnostic Sciences, University of Tennessee, Knoxville, TN, USA, [5]Mathematics, University of Tennessee, Knoxville, TN, USA, [6]National Institute for Mathematical and Biological Synthesis, [illegible]
051P	Evaluation of Diamond V Original XPC for reducing cecal colonization by Salmonella Enteriditis in layer pullets	**M. Ibukic**[1], D. Trampel[2], T. Frana[2], C.M. Logue[1], J. Broomhead[3]; [1]Department of Veterinary Microbiology and Preventive Medicine, Iowa State University, Ames, IA, USA, [2]Department of Veterinary Diagnostic and Production Animal Medicine, Iowa State University, Ames, IA, USA, [3]Diamond V, Cedar Rapids, IA, USA
052P	Bovine-deer-waterfowl interactions and Salmonella spp. transference	**D.R. Blaschka**, J. Funk, A. Mergener; Department of Large Animal Clinical Sciences, Michigan State University - College of Veterinary Medicine, East Lansing, MI, USA.
053P	Antimicrobial susceptibility of *Escherichia coli* and *Salmonella* isolated from feedlot cattle: a NARMS pilot study.	S.A. Ison[1], G.H. Loneragan[1], **B.C. Meiwes**[1], S.J. Trojan[1], J.J. Ison[1], M.M. Brashears[1], H.M. Scott[2], P. McDermott[3], S. Ayers[3], M. Torrence[4]; [1]Animal and Food Science, Texas Tech University, Lubbock, TX, USA, [2]Kansas State University, Manhattan, KS, USA, [3]FDA/CVM, Laurel, MD, USA, [4]USDA/ARS, Beltsville, MD, USA.

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FOOD AND ENVIRONMENTAL SAFETY POSTERS
Poster Session II - Monday 5:00 - 6:30 PM - Grand Ballroom Salon III - 7th floor
Section Leader: Yvette Johnson-Walker

No.	Title	Authors
054P	Shedding of foodborne pathogens and microbial carcass contamination of hunted wild ruminants	T. Obwegeser, R. Stephan, **C. Zweifel**; Institute for Food Safety and Hygiene, Vetsuisse Faculty, University of Zurich, Zurich, Switzerland.
055P	Frequency of Escherichia coli O157:H7 SNP genotypes in different cattle production systems, seasons, and sample types	W. Jung, M. Davis, **T. Besser**; Washington State University, Pullman, WA, USA.
057P	Prevalence and characterisation of CTX-M beta-lactamases amongst ExPEC from humans, companion animals, food-producing animals and retail meats in China	**J. Sun**, X. Liao, Y. Liu; Laboratory of Veterinary Pharmacology, South China Agricultural University, GuangZhou, China.
058P	The prevalence, and characterization of shiga-toxin producing *escherichia coli* (stec) serotypes from feedlot and range cattle in the us midwest.	J. Tofteland, D. Landblom, D. Doetkott, R. Gemmeda, M. Muleme, S. Olet, **M.L. Khaitsa**; Veterinary & Microbiological Sciences, North Dakota State University, Fargo, ND, USA.
059P	A Meta-analysis of the association of *Lactobacillus acidophilus* NP51 administration with *Escherichia coli* O157 in feces and on hides of feedlot cattle.	**J.J. Ison**[1], G.H. Loneragan[1], G.E. Erickson[2], R.A. Moxley[2], D.R. Smith[2], M.M. Brashears[1]; [1]Animal and Food Sciences, Texas Tech University, Lubbock, TX, USA, [2]University of Nebraska-Lincoln, Lincoln, NE, USA.

GASTROENTERIC DISEASES POSTERS
Poster Session I - Sunday 6:30-8:00 PM - Grand Ballroom Salon III - 7th floor
Section Leaders: Radhey S. Kaushik and David H. Francis
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
060P	Case-control assessment of microbiological etiology associated with calf diarrhea in Midwest USA	**Y.-I. Cho**[1], J.-I. Han[2], C. Wang[3], V. Cooper[3], K. Schwartz[3], T. Engelken[3], K.-J. Yoon[3]; [1]National Institute of Animal Science, Cheonan, Korea, Republic of, [2]College of Veterinary Medicine, Chungbuk National University, Cheongju, Korea, Republic of, [3]Department of Veterinary Diagnostic and Production Animal Medicine, Iowa State University, Ames, IA, USA.
061P	Phenotype array comparison of highly divergent *Clostridium difficile* strains	J. Scaria[1], J.-W. Chen[1], C. Mao[2], B. Sobral[2], **Y.-F. Chang**[1]; [1]Population Medicine and Diagnostic Sciences, Cornell. University, Ithaca, NY, USA, [2]Cyberinfrastructure Division, Virginia Bioinformatics Institute, Virginia Tech, Blacksburg,, VA, USA.

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GASTROENTERIC DISEASES POSTERS
Poster Session I - Sunday 6:30-8:00 PM - Grand Ballroom Salon III - 7th floor
Section Leaders: Radhey S. Kaushik and David H. Francis

No.	Title	Authors
062P	Establishment of transcriptome landscape of multiple Clostridium difficile strains using RNA sequencing	J. Scaria[1], J.-W. Chen[1], C. Mao[2], B. Sobral[2], **Y.-F. Chang**[1]; [1]Population Medicine and Diagnostic Sciences, Cornell. University, Ithaca, NY, USA, [2]Cyberinfrastructure Division, Virginia Bioinformatics Institute, Virginia Tech, Blacksburg,, VA, USA
063P	Proteomic comparison of historic and recently emerged hypervirulent Clostridium difficile strains	J.-W. Chen[1], J. Scaria[1], C. Mao[2], B. Sobral[2], **Y.-F. Chang**[1]; [1]Population Medicine and Diagnostic Sciences, Cornell. University, Ithaca, NY, USA, [2]Cyberinfrastructure Division, Virginia Bioinformatics Institute, Virginia Tech, Blacksburg,, VA, USA
064P	Immune response and protective efficacy of live attenuated Salmonella vaccine expressing antigens of Mycobacterium avium subsp. paratuberculosis against challenge in mice	S.M. Faisal[1], J.-W. Chen[1], S.P. McDonough[2], B.L. Akey[1], **Y.-F. Chang**[1]; [1]Population Medicine and Diagnostic Sciences, Cornell. University, Ithaca, NY, USA, [2]Biomedical Sciecnes, Cornell. University, Ithaca, NY, USA
065P	Potential new novel *in vitro* model for long term study of *Mycobacterium avium* ssp. *paratuberculosis* infections	**G. Kimsawatde**, N. Sriranganathan; VA-MD Regional College of Veterinary Medicine, Blacksburg, VA, USA.
066P	Adhesion to and invasion of bovine and human colonic epithelial cells by non-O157 Shiga toxin-producing Escherichia coli	**Z. Stromberg**, R. Moxley; University of Nebraska - Lincoln, Lincoln, NE, USA.
067P	Targeting *Salmonella* essential genes with antisense peptide nucleic acid	**M.A. Soofi**, M.N. Seleem; Comparative Pathobiology, Purdue University, West Lafayette, IN, USA.
068P	The iron-sulfur protein Cj0369c contributes to the aerotolerance of Campylobacter jejuni	**L. Dai**, Z. Shen, Z. Wu, Q. Zhang; Veterinary Microbiology and Preventive Medicine, Iowa State University, Ames, IA, USA.

IMMUNOLOGY POSTERS
Poster Session II - Monday 5:00 - 6:30 PM - Grand Ballroom Salon III - 7th floor
Section Leader: Laura C. Miller

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
069P	Effects on lymphocyte subpopulations of ionized alkali mineral complex-containing diets in porcine reproductive and respiratory syndrome virus infected pigs	**S. Hwang**[1], S. Kim[2], J. Song[1], H. Lee[3], T. Kim[3], Y. Park[1], S. Choi[4], B. Yoo[3], J. Han[2]; [1]Veterinary microbiology, Seoul National University, Seoul, Korea, Republic of, [2]Veterinary Medicine, Kangwon National University, Chunchon, Korea, Republic of, [3]Agribrands Purina Korea, Inc., Gyeonggi-do, Korea, Republic of, [4]BARODON-SF, Gyeonggi-do, Korea, Republic of
070P	Porcine macrophage Cdelta2+ and Cdelta2- cell lines support influenza virus infection and replication and Cdelta2+ cells mount innate immune responses to influenza virus infection.	J. Joseph[1], L. Zhu[2], C.G. Chitko-McKown[3], F. Li[1], **R.S. Kaushik**[1]; [1]Biology and Microbiology, and Veterinary and Biomedical Sciences, South Dakota State University, Brookings, SD, USA, [2]Veterinary and Biomedical Sciences, South Dakota State University, Brookings, SD, USA, [3]U.S. Meat Animal Research Center, USDA, ARS, Clay Center, NE, USA
071P	Serological surveillance of vesicular stomatitis and swine vesicular disease in korea	**H.-J. Kim**, Y.-J. Kim, H.-S. Lee, Y.-J. Ko, J.-S. Choi, J.-Y. Lee, I.-S. Cho; Animal,Plant and Fisheries Quarantine and Inspection Agency, Anyang, Korea, Republic of.
072P	Development of an epitope-based vaccine against swine influenza A virus using *Escherichia coli* heat-labile toxin B subunit as a carrier-adjuvant	Z. Sun[1], S. Lawson[1], R. Langenhorst[1], K.L. McCormick[2], C. Brunick[2], T. Opriessnig[3], R. Baker[3], K.-J. Yoon[3], W. Zhang[1], V.C. Huber[2], **Y. Fang**[1]; [1]Department of Veterinary and Biomedical Sciences, South Dakota State University, Brookings, SD, USA, [2]Division of Basic Biomedical Sciences, Sanford School of Medicine, The University of South Dakota, Vermillion, SD, USA, [3]Department of Veterinary Diagnostic and Production Animal Medicine, College of Veterinary Medicine, Iowa State University, Ames, IA, USA.
073P	Impact of oral meloxicam on circulating physiological parameters in beef steers after long distance transportation	**N. Van Engen**[1], J. Lawrence[1], T. Engelken[1], R. Vann[2], J. Sparks[1], D. Day[1], L. Karriker[1], J. Lakritz[3], W. Hsu[4], W.D. Busby[5], L. Wulf[1], J.F. Coetzee[1]; [1]VDPAM, Iowa State University, Ames, IA, USA, [2]Brown Loam Research Facility, Mississippi State University, Raymond, MS, USA, [3]Ohio State University, Columbus, OH, USA, [4]BMS, Iowa State University, Ames, IA, USA, [5]Tri-County Steer Carcass Futurity Cooperation, Tabor, IA, USA.
074P	Comparison of P2X7 receptor antagonists with bovine cells	M. Orr, R. Patel, M. Su, **D. McClenahan**; Biology, University of Northern Iowa, Cedar Falls, IA, USA.

IMMUNOLOGY POSTERS
Poster Session II - Monday 5:00 - 6:30 PM - Grand Ballroom Salon III - 7th floor
Section Leader: Laura C. Miller

No.	Title	Authors
075P	Staphylococcus aureus inhibition of dendritic cell apoptosis	**A. Johnson**, M. Lehtimaki, W. Wark, S. Neal, I. Mullarky; Virginia Tech, Blacksburg, VA, USA.
076P	Combination DNA plus protein *Brucella canis* vaccine	**H.-K. Lee**, J.-W. Kim, K. Lee, D. Kim, S.-I. Kang, S.-R. Sung, Y. Kim, J.-Y. Kim, M. Her, S.-C. Jung; Bacterial disease division, Animal, Plant and Fisheries Quarantine and Inspection Agency, Anyang, Korea, Republic of.
077P	Macrophage extracellular trap formation in response to M. haemolytica or its LKT is altered by co-incubation with bovine herpes virus-1 infected bronchiolar epithelial cells	**C. Olson**[1], K.E. Kleinow[1], N. Sennakayala[2], C.J. Czuprynski[2], N.A. Aulik[3]; [1]Biology, Winona State University, Winona, MN, USA, [2]Pathobiological Sciences, University of Wisconsin-Madison, Madison, WI, USA, [3]Pathobiological Sciences, University of Wisconsin-Madison, Madison, WI; and, Biology, Winona State University, Winona, MN, USA.
078P	*Brucella abortus* recombinant outer membrane proteins induce clearance immunity against virulent challenge in BALB/c mice.	**G.P. Andrews**[1], J.A. Leonhardt[1], A.M. Dougherty[1], J.E. Lowry[2], R. Bowen[3]; [1]Department of Veterinary Sciences, University of Wyoming, Laramie, WY, USA, [2]Department of Clinical Investigation, Eisenhower Army Medical Center, Fort Gordon, GA, USA, [3]College of Veterinary Medicine and Biomedical Sciences, Colorado State University, Fort Collins, CO, USA.
079P	Granzyme B release is triggered by activation of bovine lymphocytes	**M.K. Lehtimaki**, S. DaCosta, A. Johnson, I.K. Mullarky; Department of Dairy Science, Virginia Tech, Blacksburg, VA, USA.
080P	Optimization of 6 hours intracellular cytokine flow cytometric assay using ESAT-6-CFP-10 for diagnosis of bovine tuberculosis in Egypt	**G.S. Abdellrazeq**[1], M.M. El-Naggar[1], W.C. Davis[2], M. Singh[3]; [1]Microbiology, Faculty of Veterinary Medicine, Alexandria University, Edfina, Rosetta-line, Egypt, [2]Department of Veterinary Microbiology and Pathology, College of Veterinary Medicine,, Pullman, WA, USA, [3]Department of Genome Analysis, Helmholtz Centre for Infection Research, Braunschweig, Germany.
081P	The effect of maternal colostral immune cells on neonatal health and immune development.	**S.M. Neal**; Dairy Science, Virginia Polytechnic Institute and State University, Blacksburg, VA, USA.
082P	Association between interferon gamma production and natural resistance in *Mycobacterium bovis* naturally infected cattle.	A. Sanchez-Lopez[1], S. Flores-Villalva[1], J. Campuzano-Granados[2], **J.A. Gutierrez-Pabello**[1]; [1]Laboratorio de Investigación en Tuberculosis y Brucelosis, Facultad de Medicina Veterinaria y Zootecnia, Universidad Nacional Autónoma de México, Mexico City, Mexico, [2]Departamento de Patología, Facultad de Medicina Veterinaria y Zootecnia, Universidad Nacional Autónoma de México, Mexico City, Mexico.

IMMUNOLOGY POSTERS
Poster Session II - Monday 5:00 - 6:30 PM - Grand Ballroom Salon III - 7th floor
Section Leader: Laura C. Miller

No.	Title	Authors
083P	A novel diagnostic tool for horses with pituitary pars intermedia dysfunction (PPID)	**A.A. Adams**[1], M.H. Siard[1], K.L. Urschel[2], L. Mastro[2], D.W. Horohov[1]; [1]Veterinary Science, The Gluck Equine Research Center, Lexington, KY, USA, [2]Animal and Food Sciences, University of Kentucky, Lexington, KY, USA.
084P	Comparison of nutritional compounds (pterostilbene, resveratrol, curcuminoids, quercetin, and hydroxypterostilbene) to NSAIDs on equine cytokine production in vitro	M.H. Siard, K.E. McMurry, D.W. Horohov, **A.A. Adams**; Veterinary Science, The Gluck Equine Research Center, Lexington, KY, USA.
085P	Immunogenic ability of a recombinant QseC, a bacterial adrenergic receptor, to induce innate and adaptive immune responses in avian macrophages	**A.A. Chaudhari**, S. Kariyawasam; Department of Veterinary and Biomedical Sciences, The Pennsylvania State University, State college, PA, USA.
086P	Magnetic resonance microscopic imaging of hearts reveals structural and functional defects in autoimmune myocarditic mice.	**C. Massilamany**[1], V. Khalilzad[2], A. Gangaplara[1], D. Steffen[1], S.F. Othman[2], J. Reddy[1]; [1]School of Veterinary Medicine and Biomedical Sciences, University of Nebraska-Lincoln, Lincoln, NE, USA, [2]Department of Biological Systems Engineering, University of Nebraska-Lincoln, Lincoln, NE, USA.

RESPIRATORY DISEASES POSTERS
Poster Session II - Monday 5:00 - 6:30 PM - Grand Ballroom Salon III - 7th floor
Section Leaders: Amelia Woolums and Christopher 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
087P	The use of a new porcine epithelial cell model for the study of PRRSV-PCV co-infection reveals a PCV genotype dependent effect	**F. Alvarez**, C. Provost, C. Savard, C.A. Gagnon; Faculty of Veterinary Medicine, Université de Montréal, Saint-Hyacinthe, QC, Canada.
088P	Development of an immortalized canine respiratory epithelial cell line for canine influenza virus infection	**I.-S. Choi**, W.-J. Park, Y.-J. Song, J.-B. Lee, S.-Y. Park, C.-S. Song, N.-H. Lee; Infectious diseases, Konkuk University, College of Veterinary Medicine, Seoul, Korea, Republic of.
089P	Protection effect against PRRSV infection and boosting effect on PRRSV vaccine of immunostimulator(Barodon®) in pigs	**S.J. Kim**[1], B.W. Yoo[2], S.I. Choi[3], S.Y. Hwang[4], J.H. Han[1]; [1]College of Veterinary Medicine and Institute of Veterinary Science, Kangwon national university, Chuncheon, Korea, Republic of, [2]Cargill agri purina, Sungnam, Korea, Republic of, [3]Barodon SF, Ansung, Korea, Republic of, [4]Microbiology Lab., Seoul national university, Seoul, Korea, Republic of.
090P	Barn dust exposure impairs swine alveolar macrophage function:implications for swine respiratory health	S.M. Knetter[1], **C.K. Tuggle**[1], M.J. Wannemuehler[2], A. Ramer-Tait[2]; [1]Animal Science, Iowa State University, Ames, IA, USA, [2]Veterinary Microbiology and Preventive Medicine, Iowa State University, Ames, IA, USA.

RESPIRATORY DISEASES POSTERS
Poster Session II - Monday 5:00 - 6:30 PM - Grand Ballroom Salon III - 7th floor
Section Leaders: Amelia Woolums and Christopher Chase

No.	Title	Authors
091P	*In vitro* biofilm formation by *Mannheimia haemolytica*	**I. Boukahil**, K. Brandenburg, C. Czuprynski; University of Wisconsin-Madison, Madison, WI, USA.
092P	The kinetics of white blood cell counts during vaccination against Bovine Respiratory Disease pathogens and their correlations with lung lesions, diagnosis and average daily gain.	R.J. Leach[1], **C.G. Chitko-McKown**[2], L.A. Kuehn[1]; [1]Genetics & Breeding Research Unit, U.S. Meat Animal Research Center, Clay Center, NE, USA, [2]Animal Health Research Unit, U.S. Meat Animal Research Center, Clay Center, NE, USA.

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
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
093P	Avian hemoparasites in Illinois and their effects on health	**K.L. Annetti**; Wildlife Disease, Illinois Natural History Survey, Champaign, IL, USA.
094P	Detection of *Bartonella* species from cattle ticks in South Korea	**J.-Y. Kim**, M. Chae, S.-I. Kang, M. Her, J. Gu, H. Lee, K. Lee, Y. Ha, S. Kang, S. Jung, S. Choe; Bacterial disease division, Animal, plant and fisheries Quarantine and Inspection Agency, Anyang, Korea, Republic of.
095P	Effect of skin lesion on Haematological picture of some dogs in Ibadan.	I.A Adetiba; University of Ibadan, Nigeria, Ibadan, Nigeria.

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
096P	Clathrin-mediated endocytosis is required for porcine epidemic diarrhea virus entry into Vero cells	**H. Shin**, J.-E. Park; Laboratory of Infectious Diseases, College of Veterinary Medicine, Chungnam National University, Taejon, Korea, Republic of.
097P	Effectiveness of small interfering RNA (siRNA) to inhibit feline coronavirus replication	**E.A. Anis**[1], R.P. Wilkes[1], S.A. Kania[1], A. Legendre[2], M. Kennedy[1]; [1]Biomedical and Diagnostic Sciences, University of Tennessee, Knoxville, TN, USA, [2]Small Animal Clinical Sciences, University of Tennessee, Knoxville, TN, USA
098P	Construction and characterization of infectious clone of an interferon-inducing PRRSV strain	**Y. Yu**, R. Wang, Y. Nan, Y. Zhang; Molecular Virology Laboratory, VA-MD Regional College of Veterinary Medicine, University of Maryland, College Park, MD, USA.
099P	The nonstructural protein 1 of murine arterivirus lactate dehydrogenase elevating virus is a viral type I interferon antagonist	**M. Han**, Y. Sun, C. Kim, D. Yoo; Department of Pathobiology, University of Illinois at Urbana-Champaign, Urbana, IL, USA.
100P	Biological properties of low pathogenic influenza A viruses isolated from wild birds in the Black Sea region of Ukraine	**D. Muzyka**[1], B. Stegniy[2], A. Stegniy[1]; [1]Avian Diseases Epizootology, National Scientific Center Institute of Experimental and Clinical Veterinary Medicine, Kharkiv, U, Kharkiv, Ukraine, [2]Avian Diseases, National Scientific Center Institute of Experimental and Clinical Veterinary Medicine, Kharkiv, U, Kharkiv, Ukraine.
101P	Phylogenetic studies of Ukrainian NDV isolates	**V.I. Bolotin**[1], A.P. Gerilovych[1], O.S. Solodiankin[1], D.V. Muzyka[1], C.L. Afonso[2]; [1]National Scientific Center Institute of Experimental and Clinical Veterinary Medicine, Kharkiv, Ukraine, [2]United States Department of Agriculture, Southeast Poultry Research Laboratory, Athens, GA, USA.
102P	Mutation in noncytopathic BVDV persistently infected animals to generate cytopathic pair is a rare event where one animal developed a cytopathic virus that hit all the animals within one herd.	**M.F. Darweesh**[1], J. Ridpath[2], J. Neil[2], A. Young[1], L. Braun[1], M. Rajput[1], C. Chase[1]; [1]Vet., and biomedical science, South Dakota State University, Brookings, SD, USA, [2]Agricultural Research Service, United States Department of Agriculture,, Ruminant Diseases and Immunology Research Unit, National Animal Disease Center, Ames, IA, USA

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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

No.	Title	Authors
103P	Non adherent cd14 negative bovine monocyte derived dendritic lose their capability to produce infectious bovine viral diarrhea virus (bvdv) during its development	**M.K.S. Rajput**[1], L.J. Braun[1], M.F. Darweesh[1], J.F. Ridpath[2], W. Mwangi[3], A. Young[1], C.C.L. Chase[1]; [1]Department of Veterinary and Biomedical Sciences, South Dakota State University, Brookings, SD, USA, [2]Ruminant Diseases and Immunology Research Unit, National Animal Disease Center, Agricultural Research Service, United States Department of Agriculture, Ames, IA, USA, [3]Department of Veterinary Pathobiology, Texas A&M University, College Station, TX, USA.
104P	Genetic variability of bovine diarrhea pestiviruses, detected in semen and veterinary drugs	**A.P. Gerilovych**, A.B. Stegniy, I.V. Goraichuk, V.I. Bolotin, R.O. Kucheryavenko; Molecular epidemiology and diagnostics, NSC Institute of experimental and clinical veterinary medicine, Kharkiv, Ukraine.
105P	Parvovirus detection in feline feces via pcr	**B.E. Thiel**, L.J. Larson, R.D. Schultz; Pathobiological Sciences, University of Wisconsin-Madison, Madison, WI, USA.
106P	Will pcr detect antibody-neutralized cdv and cpv-2 virus? do storage conditions affect pcr ct values?	B.E. Thiel[1], **L.J. Larson**[1], K. Kurth[2], R.D. Schultz[1]; [1]Pathobiological Sciences, University of Wisconsin-Madison, Madison, WI, USA, [2]Wisconsin Veterinary Diagnostic Laboratory, Madison, WI, USA.
107P	Nanoparticle delivery of siRNAs into feline cells in vitro	**R.P. Wilkes**[1], M.E. Hall[1], S. Tang[2], S.C. Lenaghan[3], W. He[2]; [1]Biomedical and Diagnostic Sciences, The University of Tennessee College of Veterinary Medicine, Knoxville, TN, USA, [2]Materials Science and Engineering, The University of Tennessee, Knoxville, TN, USA, [3]Mechanical, Aerospace, and Biomedical Engineering, The University of Tennessee, Knoxville, TN, USA.

ORAL PROGRAM

BACTERIAL PATHOGENESIS
Avenue Ballroom - 4th Floor
Section Leader: Gireesh Rajashekara

		Presiders: Adel Talaat and Gireesh Rajashekara	
Time	**No.**	**Title**	**Authors**
8:00 Mon.	**001**	Inhibition of Pseudomonas aeruginosa biofilm formation on a biological wound dressing	**K.S. Brandenburg**[1], J.F. McAnulty[2], C.J. Murphy[3], N.L. Abbott[4], M.J. Schurr[5], C.J. Czuprynski[1]; [1]Pathobiological Sciences, University of Wisconsin - Madison, Madison, WI, USA, [2]Surgical Sciences, University of Wisconsin - Madison, Madison, WI, USA, [3]Surgical and Radiological Sciences, University of California - Davis, Davis, CA, USA, [4]Chemical and Biological Engineering, University of Wisconsin - Madison, Madison, WI, USA, [5]Surgery, University of Colorado -
8:15	**002**	The role of exopolyphosphatase/ guanosine pentaphosphate phosphohydrolase (ppx/gppa) enzymes of *campylobacter jejuni*	**A. Kumar**[1], D. Gangaiah[1], K. Chandrashekhar[1], J. Arcos[2], J. Torrelles[2], G. Rajashekara[1]; [1]Food Animal Helath Research Program, The Ohio State University, Wooster, OH, USA, [2]Microbial Infection and Immunity, The Ohio State University, Columbus, OH, USA.
8:30	**003**	*Campylobacter jejuni* isolates from calves have A, B and C lipooligosaccharide (LOS) biosynthetic locus classes similar to human Guillain Barré syndrome associated strains	**J.L. St. Charles**[1], R. Mosci[2], J. Rudrik[3], S.D. Manning[2], L.S. Mansfield[2]; [1]Comparative Medicine Integrative Biology, Michigan State University, East Lansing, MI, USA, [2]Department of Microbiology and Molecular Genetics, Michigan State University, East Lansing, MI, USA, [3]Bureau of Laboratories, Department of Community Health, Michigan Department of Community Health, Lansing, MI, USA.
8:45	**004**	Distribution of virulence genes in Canadian Haemophilus parasuis strains	G.A. Soltes[1], P. Boerlin[1], Z. Poljak[2], V.M. Nicholson[1], J. Gallant[3], **J.I. MacInnes**[1]; [1]Dept. of Pathobiology, University of Guelph, Guelph, ON, Canada, [2]Dept. of Population Medicine, University of Guelph, Guelph, ON, Canada, [3]Gallant Custom Laboratories, Cambridge, ON, Canada
9:00 Mon.	**005**	Evaluation of invasion by nonpathogenic *Salmonella enterica* serovar Kentucky in poultry intestinal epithelia cells.	**K. Howe**[1], H. Bailey[1], M. Lawrence[2], A. Karsi[2], J. Brooks[3], R. Wills[1]; [1]Department of Pathobiology and Population Medicine, Mississippi State University College of Veterinary Medicine, Mississippi State, MS, USA, [2]Basic Science Department, Mississippi State University College of Veterinary Medicine, Mississippi State, MS, USA, [3]USDA ARS Genetics and Precision Agriculture, United States Department of Agriculture, Mississippi

BACTERIAL PATHOGENESIS
Avenue Ballroom - 4th Floor
Section Leader: Gireesh Rajashekara

		Presiders: Adel Talaat and Gireesh Rajashekara	
Time	**No.**	**Title**	**Authors**
9:15 Mon.	**006**	Comparative transcriptome analysis using RNA-seq reveals differences in global gene expression profiles between high-pahtogenic and low-pathogenic *Salmonella* Enteritidis strains	**D.H. Shah**; Department of Veterinary Microbiology and Pathology, Washington State University, Pullman, WA, USA.
9:30		**Break and Table Top Exhibits – Foyer**	
		Presiders: Timothy Johnson and Raul Almeida	
10:00	**007**	Sequence of two plasmids from Clostridium perfringens chicken necrotic enteritis isolates and comparison with C. perfringens conjugative plasmids	**J. Prescott**[1], V.R. Parreira[1], M. Costa[1], F. Eikmeyer[2], J. Blom[3]; [1]Pathobiology, University of Guelph, Guelph, ON, Canada, [2]Institute for Genome Research and Systems Biology, Center for Biotechnology, Bielefeld University, Bielefeld, Germany, [3]Bioinformatics Resource Facility, Center for Biotechnology, Bielefeld University, Bielefeld, Germany.
10:15 Mon.	**008**	Comparative genome analysis of an avirulent and two virulent strains of avian *Pasteurella multocida*	**T.J. Johnson**[1], J. Abrahante[1], S.S. Hunter[2], F.M. Tatum[3], S.K. Maheswaran[1], R.E. Briggs[3]; [1]Veterinary and Biomedical Sciences, University of Minnesota, Saint Paul, MN, USA, [2]Institute for Bioinformatics and Evolutionary Studies, University of Idaho, Moscow, ID, USA, [3]NADC, ARS, USDA, Ames, IA, USA.
10:30	**009**	Host specificity in *Pasteurella multocida*	**T.J. Johnson**[1], J.E. Abrahante[1], S.S. Hunter[2], F.M. Tatum[3], S.K. Maheswaran[1], R.E. Briggs[3]; [1]Veterinary and Biomedical Sciences, University of Minnesota, Saint Paul, MN, USA, [2]Institute for Bioinformatics and Evolutionary Studies, University of Idaho, Moscow, ID, USA, [3]NADC, ARS, USDA, Ames, IA, USA.
10:45-11:30 Keynote	**010**	**Keynote**: Roles of type IV secretion system in obligatory intracellular infection.	**Y. Rikihisa**, H. Niu, H. Liu, M. Lin, Q. Xiong; Veterinary Biosciences, The Ohio State University, Columbus, OH, USA.
11:30		**Lunch Break**	
		Presiders: Gireesh Rajashekara	
1:15 Mon.	**011**	Evaluation of bovine neutrophil activation by *Leptospira*	**J. Wilson-Welder**, D. Alt; Infectious Bacterial Disease of Livestock, National Animal Disease Center, ARS-USDA, Ames, IA, USA.

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BACTERIAL PATHOGENESIS
Avenue Ballroom - 4th Floor
Section Leader: Gireesh Rajashekara

		Presiders: Gireesh Rajashekara	
Time	**No.**	**Title**	**Authors**
1:30 Mon.	**012**	Lymphocyte subpopulations influence murine susceptibility to the agent of epizootic bovine abortion.	**M.T. Blanchard**[1], C.I. Chen[2], M. Anderson[3], B.V. Yeargan[1], M. Hall[4], J.L. Stott[1]; [1]Vet Med: Pathology, Microbiology and Immunology, University of California, Davis, CA, USA, [2]Dept. of Pathology, Northwestern University, Chicago, IL, USA, [3]California Animal Health and Food Safety System, University of California, Davis, CA, USA, [4]Professor Emeritus, University of Nevada, Reno, NV, USA.
1:45	**013**	Challenge study to assess association between Moraxella bovoculi and Infectious bovine Keratoconjunctivitis in calves	S. Gould, R. Dewell, K. Tofflemire, D. Whitley, S. Millman, T. Opriessnig, R. Rosenbusch, **A. O'Connor**; Iowa State University, Ames, IA, USA.
2:00	**014**	Comparison of induced small animal models for Guillain Barré syndrome (GBS) as post infectious sequelae to *Campylobacter jejuni* infection	**L.S. Mansfield**[1], J.L. St. Charles[2], B.J. Gadsden[2], A. Malik[1], H.Y. Kim[1], J.A. Bell[1]; [1]Department of Microbiology and Molecular Genetics, Michigan State University, East Lansing, MI, USA, [2]Comparative Medicine Integrative Biology, Michigan State University, East Lansing, MI, USA.
2:15	**015**	Cellulutis in turkeys and the role of gut integrity	**A.J. Thachil**, K.V. Nagaraja; Veterinary and Biomedical Sciences, University of Minnesota, Saint Paul, MN, USA.
2:30	**016**	Optimization of *in vitro* growth conditions and DNA extraction from T*reponema phagedenis* isolated from bovine digital dermatitis lesions.	**A. Krull**, P. Plummer; Veterinary Diagnostic & Production Animal Medicine, Iowa State University, Ames, IA, USA.
2:45		**Break and Table Top Exhibits – Foyer**	
3:00 Mon.	**017**	Use of anti-SUAM antibodies in a Passive protection model to prevent Streptococcus uberis mastitis	**R.A. Almeida**[1], O. Kerro-Dego[1], S.I. Headrick[1], M.J. Lewis[2], C. Young[2], B.E. Gillespie[1], L.S. Siebert[1], D.A. Luther[1], G.M. Pighetti[1], S.P. Oliver[1]; [1]Animal Science, The University of Tennessee, Knoxville, TN, USA, [2]East Tennessee AgResearch and Education Center-Little River Animal and Environmental Unit, The University of Tennessee, Knoxville, TN, USA.
3:15	**018**	Defining the role of SUAM in the pathogenesis of Streptococcus uberis mastitis using a SUAM-negative gene deletion mutant	**R.A. Almeida**[1], O. Kerro-Dego[1], S.I. Headrick[1], M.J. Lewis[2], C. Young[2], B.E. Gillespie[1], L.S. Siebert[1], D.A. Luther[1], G.M. Pighetti[1], S.P. Oliver[1]; [1]Animal Science, The University of Tennessee, Knoxville, TN, USA, [2]East Tennessee AgResearch and Education Center-Little River Animal and Environmental Unit, The University of Tennessee, Knoxville, TN, USA.

BACTERIAL PATHOGENESIS
Avenue Ballroom - 4th Floor
Section Leader: Gireesh Rajashekara

		Presiders: Gireesh Rajashekara	
Time	**No.**	**Title**	**Authors**
3:30 Mon.	**019**	Transcriptome expression profiles of Streptococcus uberis during bovine mastitis	O. Kerro-Dego[1], S.P. Oliver[1], A.M. Saxton[1], L.J. Hauser[2], **R.A. Almeida**[1]; [1]Animal Science, The University of Tennessee, Knoxville, TN, USA, [2]Computational Biology and Bioinformatics Group, Oak Ridge National Labs, Oak Ridge, TN and Dept. of, The University of Tennessee, Knoxville, TN, USA.
3:45	**020**	Next-generation sequencing of Streptococcus uberis UT888 genome facilitates quest for virulence /pathogenic associated gene features	**R.A. Almeida**[1], D.A. Luther[1], O. Kerro-Dego[1], S.A. Kania[2], L. Hauser[3], A.M. Saxton[1], S.P. Oliver[1]; [1]Animal Science, The University of Tennessee, Knoxville, TN, USA, [2]Dept. of Comparative Medicine, University of Tennessee College of Veterinary Medicine, The University of Tennessee, Knoxville, TN, USA, [3]Computational Biology and Bioinformatics Group, Oak Ridge National Labs, Oak Ridge, TN and Dept. of, The University of Tennessee, Knoxville, TN, USA
4:00	**021**	Mechanisms of intrinsic resistance to antimicrobial peptides of Edwardsiella ictaluri and its influence on fish gut inflammation and virulence.	**J. Santander**, T. Martin, A. Loh, R. Curtiss; Arizona State University, Tempe, AZ, USA.
4:15 Mon.	**022**	Penicillin-binding proteins and cefoxitin in Staphylococcus pseudintermedius and Staphylococcus schleiferi subspecies coagulans	**D.V. Diaz-Campos**, K.V. Brock, T. Hathcock; Biomedical Sciences, Pathobiology., Auburn University, Auburn, AL, USA.
4:30 to	**5:00**	**Break and Table Top Exhibits – Foyer**	
5:00 to	**6:30**	**Poster Session II Grand Ballroom Salon III - 7th floor**	

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

Time	No.	Title	Authors
11:30		**Lunch Break**	
1:30 Mon.	**023**	Carriage probability of avian influenza viruses in wild waterfowl influenced by host and environmental factors	**R. Ivanek**[1], S. Zhang[2], B. Szonyi[1], I. Srinath[1], S.-S. Park[1], P. Ferro[3], B. Lupiani[3], M. Peterson[4], J. Huang[2], R. Carroll[2]; [1]Veterinary Integrative Biosciences, Texas A&M University, College Station, TX, USA, [2]Dept. of Statistics, Texas A&M University, College Station, TX, USA, [3]Veterinary Pathobiology, Texas A&M University, College Station, TX, USA, [4]Wildlife and Fisheries Sciences, Texas A&M University, College Station, TX, USA.
1:45	**024**	Electronic microarrays for detection and typing of high consequence agents in swine	**A. Ambagala**[1], O. Lung[1], D. Hodko[2], J. Pasick[3], Z. Zhang[3], D. King[4], T. Furukawa-Stoffer[1], S. Ohene-Adjei[1], K. Burton Hughes[1], M. Fisher[1], C. Buchanan[1]; [1]National Centres for Aniimal Disease, Canadian Food Inspection Agency, Lethbridge, AB, Canada, [2]Nexogen Inc., San Diego, CA, USA, [3]National Centres for Aniimal Disease, Canadian Food Inspection Agency, Winnipeg, MB, Canada, [4]Institute for Animal Health, Pirbright, Surrey, UK.
2:00	**025**	Serotype reactivity of commercial immunoassays for *Salmonella enterica* identification in experimentally-inoculated equine fecal samples	**B.A. Burgess**, D.S. Bolte, D.R. Hyatt, D.C. Van Metre, P.S. Morley; Clinical Sciences, Colorado State University, Fort Collins, CO, USA.
2:15	**026**	Environmental survival of Equid Herpesvirus -1.	**N.T. Saklou**, L.V. Ashton, L.S. Goehring; Clinical Sciences, Colorado State University, Fort Collins, CO, USA.
2:30 Mon.	**027**	Efficacy of Sodium Dodecyl Sulfate and Formic acid inactivation of Caprine Arthritis-Encephalitis virus in vitro	A. Morales-delaNuez[1], P. Plummer[2], S. Hartmann[3], P. Nara[4], A. Argüello[1], J**. Trujillo**[4]; [1]Universidad de Las Palmas de Gran Canaria, Arucas, Spain, [2]ISU-VMPM, Ames, IA, USA, [3]Drexel University, Philadelphia, PA, USA, [4]ISU-CAHDIT, ames, IA, USA.
2:45		**Break and Table Top Exhibits – Foyer**	
3:00-3:45 Keynote	**028**	**Keynote:** African Swine Fever: Current Situation and Control Strategy	**A.D. Zaberezhny**; D. I. Ivanovski Virology Institute, Moscow, Russian Federation.
3:45	**029**	Complying with U.S. export controls as a life science researcher	**K.A. Orr**; Bureau of Industry and Security, Dept of Commerce, Washington, DC, USA.

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

Time	No.	Title	Authors
4:00 Mon.	**030**	Development and implementation of an HSEEP compliant avian influenza response training exercise for zoological personnel.	**M. Myint**[1], Y.J. Johnson[1], Y. Nadler[2], E. Field[3], M. Ruiz[4], J. Kunkle[3], A. Ruaman[5]; [1]Veterinary Clinical Medicine, UIUC, College of Veterinary Medicine, Urbana, IL, USA, [2]Lincoln Park Zoo, Chicago, IL, USA, [3]Illinois Department of Agriculture, Springfield, IL, USA, [4]Veterinary Pathobiology, UIUC, College of Veterinary Medicine, Urbana, IL, USA, [5]Veterinary Services, USDA, Springfield, IL, USA.
4:15 to	**4:30**	Open	
4:30 to	**5:00**	**Break and Table Top Exhibits – Foyer**	
5:00 to	**6:30**	**Poster Session II Grand Ballroom Salon III - 7th floor**	

COMPANION ANIMAL EPIDEMIOLOGY
Denver/Houston Room - 5th Floor
Section Leader: Margaret Slater and Laura Hungerford

Time	No.	Title	Authors
		Presiders: Margaret Slater and Erin Leonard	
8:00-8:45 Tues. Keynote	**031**	**Companion Animal Epidemiology Keynote in Salon A/B/C/D Rm, 5th Floor:** From licking stamps to clicking buttons - moving from conventional questionnaires to online surveys	**M.G. Doherr**; Dept. Clin. Res. & Vet. Public Health, Vetsuisse Faculty, University of Bern, Bern-Liebefeld, Switzerland.
8:45	**032**	**Mark Gearhart Memorial Graduate Student Award in Salon A/B/C/D Rm, 5th Floor:** Nasal shedding of Equine Herpesvirus-1 from horses in an outbreak of Equine Herpes Myeloencephalopathy in Western Canada	**B.A. Burgess**1, N. Tokateloff2, K. Poirier2, S. Manning2, K. Lohmann2, D.P. Lunn3, S.B. Hussey3, P.S. Morley3; 1University of Saskatchewan, Saskatoon, Canada; and, Animal Population Health Institute, Colorado State University, Fort Collins, CO, USA, 2Large Animal Clinical Sciences, Western College of Veterinary Medicine, University of Saskatchewan, Saskatoon, SK, Canada, 3Animal Population Health Institute, Colorado State University, Fort Collins, CO, USA.
9:00	**033**	Risk factors for antimicrobial resistant *Salmonella* spp. and *Escherichia coli* carriage in pet dogs from volunteer households in Ontario (2005-2006)	**E.K. Leonard**[1], D.L. Pearl[1], N. Janecko[1], R.L. Finley[2], R.J. Reid-Smith[3], J.S. Weese[4], A.S. Peregrine[4]; [1]Population Medicine, University of Guelph, Guelph, ON, Canada, [2]Centre for Food-Borne, Environmental & Zoonotic Infectious Diseases, Public Health Agency of Canada, Guelph, ON, Canada, [3]Laboratory for Foodborne Zoonoses, Public Health Agency of Canada, Guelph, ON, Canada, [4]Pathobiology, University of Guelph, Guelph, ON, Canada.
9:15	**034**	Syndromic surveillance for nosocomial infections in small animal veterinary referral hospitals	**A. Ruple**[1], H. Aceto[2], J. Bender[3], M. Paradis[4], S. Shaw[5], D. Van Metre[1], J.S. Weese[6], D. Wilson[7], J. Wilson[3], P. Morley[1]; [1]Colorado State University, Fort Collins, CO, USA, [2]University of Pennsylvania, Kennett Square, PA, USA, [3]University of Minnesota, St. Paul, MN, USA, [4]Tufts University, Grafton, MA, USA, [5]New England Veterinary Center and Cancer Care, Windsor, CT, USA, [6]University of Guelph, Guelph, ON, Canada, [7]University of Missouri, Columbia, MO, USA
9:30		**Break and Table Top Exhibits – Foyer**	
10:00 Tues.	**035**	Survey to investigate pet ownership and attitudes to pet care in metropolitan Chicago dog and/or cat owners.	**A. Litster**, A. Freiwald; Veterinary Clinical Sciences, Purdue University, West Lafayette, IN, USA.
10:15	**036**	Birth and death rate estimates and selected owner demographic data associated with cat, dog, pet bird, and horse ownership in U.S. households in 2006	**J.C. New, Jr.**, W.J. Kelch, A.P. Golden; Biomedical and Diagnostic Sciences, University of Tennessee College of Veterinary Medicine, Knoxville, TN, USA.

COMPANION ANIMAL EPIDEMIOLOGY
Denver/Houston Room - 5th Floor
Section Leader: Margaret Slater and Laura Hungerford

Time	No.	Title	Authors
		Presiders: Margaret Slater and Erin Leonard	
10:30 Tues.	**037**	Use of survival analysis to assess the effects of fee structure on post-adoption relinquishment of dogs and cats	J.K. Levy[1], M. Fei[2], J. Willson[1], S.C. Zeidman[3], **H.M. Scott**[2]; [1]Maddie's Shelter Medicine Program, University of Florida, Gainesville, FL, USA, [2]Diagnostic Medicine and Pathobiology, Kansas State University, Manhattan, KS, USA, [3]PetHealth, Inc., Oakville, ON, Canada.
10:45	**038**	Risk factors for the development of malignant histiocytosis in Bernese Mountain Dogs	**A. Ruple**, P. Morley; Colorado State University, Fort Collins, CO, USA.
11:00	**039**	Prevalence of feline influenza virus infection in cats in Bangladesh.	**M.S. Rahman**, M.E. Alam; Medicine, Bangladesh Agricultural University, Mymensingh, Bangladesh.
11:15 Tues.	**040**	The reliability of a survey to score cat socialization from unsocialized to highly socialized	**M.R. Slater**[1], K. Miller[2], E. Weiss[3], A. Mirontschuk[4], K. Makolinski[5], L. Garrison[6]; [1]Shelter Research and Development, The American Society for the Prevention of Cruelty to Animals, Florence, MA, USA, [2]Anti-Cruelty Behavior Team, The American Society for the Prevention of Cruelty to Animals, New York, NY, USA, [3]Shelter Research and Development, The American Society for the Prevention of Cruelty to Animals, Benton, KS, USA, [4]Shelter Research and Development, The American Society for the Prevention of Cruelty to Animals, Oakland, CA, USA, [5]Veterinary Outreach, The American Society for the Prevention of Cruelty to Animals, Orchard Park, NY, USA, [6]Shelter Research and Development, The American Society for the Prevention of Cruelty to Animals, New York, NY, USA.
11:45 to	**12:30**	**Business Meeting, Dedication, New Members Introduction, and Graduate Student Competition Awards Presentations**	

EPIDEMIOLOGY AND ANIMAL HEALTH ECONOMICS
Salons A/B/C/D - 5th Floor
Section Leader: Ashley Hill

Time	No.	Title	Authors
8:00 Mon.	041	Prioritization of zoonoses in North America: A public perspective	V. Ng, **J.M. Sargeant**; Population Medicine, Ontario Veterinary College, Guelph, ON, Canada.
8:15	042	Prioritization of Zoonoses in North America: Animal and human health professionals' perspective	V. Ng, **J.M. Sargeant**; Population Medicine, Ontario Veterinary College, Guelph, ON, Canada.
8:30	043	Methicillin Resistant *Staphylococcus aureus* in Dairy Farms - Is there a need to worry?	**L. da Costa**, P.J. Rajala-Schultz, A. Hoet, J. Van Balen, G. Schuenemann; Veterinary Preventive Medicine, The Ohio State University, Columbus, OH, USA.
8:45	044	Non-tuberculous mycobacteria in the pastoral ecosystems of Uganda: "One health, One ecosystem"	**J. Oloya**[1], C. Kankya[2], E. Skjerve[3]; [1]Epidemiology and Biostatistics, College of Public Health, Athens, GA, USA, [2]Veterinary Public Health and Preventive Medicine, Makerere University, Kampala, Uganda, [3]Epidemiology and Biostatistics, Norwegian School of Veterinary Science, Oslo, Norway
9:00	045	Comparative study of the prevalence of brucellosis in cattle, goats and humans from farms in southwestern Uganda	**R. Miller**[1], J.L. Nakavuma[2], P. Ssajjakambwe[2], P. Vudriko[2], R. Musese[2], J.B. Kaneene[1]; [1]Center for Comparative Epidemiology, Michigan State University, East Lansing, MI, USA, [2]College of Veterinary Medicine, Animal Resources and Biosecurity, Makerere University, Kampala, Uganda.
9:15	046	Time series model for human and bovine brucellosis cases in South Korea between 2005 and 2010	**H.S. Lee**[1], M. Her[2], M. Levine[3], G.E. Moore[1]; [1]Department of Comparative Pathobiology, College of Veterinary Medicine, Purdue University, West Lafayette, IN, USA, [2]Animal and Plant Health Research, Animal, Plant and Fisheries Quarantine and Inspection Agency (QIA), OIE Reference Laboratory for Brucellosis, Bacterial Disease Division, 175, Anyang-ro, Manan-gu, Anyang-si, Gyeonggi, Korea, Republic of, [3]Department of Statistics, Purdue University, West Lafayette, IN, USA
9:30		**Break and Table Top Exhibits – Foyer**	
10:00 Mon.	047	The prevalence and spatial distribution of avian reovirus among Ontario broiler chicken flocks	**E. Nham**[1], M. Guerin[1], D. Ojkic[2], D. Pearl[1], D. Durda Slavic[2]; [1]Population Medicine, Ontario Veterinary College, University of Guelph, Guelph, ON, Canada, [2]Animal Health Laboratory, Laboratory Services Division, University of Guelph, Guelph, ON, Canada

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EPIDEMIOLOGY AND ANIMAL HEALTH ECONOMICS
Salons A/B/C/D - 5th Floor
Section Leader: Ashley Hill

Time	No.	Title	Authors
10:15 Mon.	048	Prevalence, characterization, and seasonal variation of *Clostridium perfringens* in Ontario broiler chicken flocks.	**H. Kasab-Bachi**[1], M. Guerin[1], S. McEwen[1], D. Pearl[1], D. Slavic[2], A. Boecker[3]; [1]Population Medicine, University of Guelph, Guelph, ON, Canada, [2]Animal Health Laboratory, Laboratory Services Division, University of Guelph, Guelph, ON, Canada, [3]Department of Food, Agricultural and Resource Economics, University of Guelph, Guelph, ON, Canada.
10:30	049	Prevalence, seasonality, and geographical distribution of chicken anemia virus, fowl adenovirus, and infectious bursal disease virus in Ontario broiler chickens.	**M.E. Eregae**[1], C. Dewey[1], S. McEwen[1], D. Ojkic[2], M. Guerin[1]; [1]Population Medicine, University of Guelph, Guelph, ON, Canada, [2]Animal Health Laboratory, Guelph, ON, Canada
10:45	050	The epidemiology of *Brachyspira* species in Ontario layer chicken flocks	**G. Medhanie**[1], S. McEwen[1], L. Weber[2], L. Cooley[3], S. Houghton[4], B. Sanei[5], D. Slavic[6], M.T. Guerin[1]; [1]Population Medicine, University of Guelph, Guelph, ON, Canada, [2]Weber Consulting Services, Guelph, ON, Canada, [3]L.H. Gray & Son, Strathroy, ON, Canada, [4]Burnbrae farms, Waterloo, ON, Canada, [5]Ontario Ministry of Agriculture, Food and Rural Affairs, Guelph, ON, Canada, [6]University of Guelph, Animal Health Laboratory, ON,
11:00	051	Post-vaccination monitoring and surveillance for Highly Pathogenic Avian Influenza in Long An Province, Vietnam, 2009: design and findings	**V.T. Le**[1], B.X. Nguyen[1], H.T. Nguyen[1], L.T. Ngo[1], L.V. Nguyen[2], T.T.T. Nguyen[3], K.T.M. Le[3], P. Padungtod[4], K. Kanachai[5], D.T.T. Phan[1], H.Q. Tran[1], P.D. Thai[1]; [1]Department Animal Health, Vietnam, Regional Animal Health Office Number VI, Hochiminh, Viet Nam, [2]Ministry of Agriculture and Rural Development, Vietnam, Department Animal Health, Hanoi, Viet Nam, [3]Long An Sub Department Animal Health, Long An, Viet Nam, [4]U.S.CDC Southeast Asia Regional Office, Global Disease Detection Regional Center, Bangkok, Thailand, [5]Department of Livestock Development, Field Epidemiology Training Program for Veterinarians (FETPV), Bangkok, Thailand.
11:15 Mon.	052	Evaluation of molecular profiling tools to differentiate strains of Salmonella Enteriditis	**M. Ibukic**[1], T. Frana[2], D. Trampel[2], C.M. Logue[1]; [1]Department of Veterinary Microbiology and Preventive Medicine, Iowa State University, Ames, IA, USA, [2]Department of Veterinary Diagnostic and Production Animal Medicine, Iowa State University, Ames, IA, USA.

EPIDEMIOLOGY AND ANIMAL HEALTH ECONOMICS
Salons A/B/C/D - 5th Floor
Section Leader: Ashley Hill

Time	No.	Title	Authors
11:30		**Lunch Break**	
1:30 Mon.	**053**	Comparison of PCR assays for reliable, early and fast detection of PRRSV in different sample types from experimentally infected boars	**P. Gerber**, K. O'Neill, O. Owolodun, C. Branstad, P. Halbur, T. Opriessnig; Department of Veterinary Diagnostic and Production Animal Medicine, Iowa State University, Ames, IA, USA.
1:45	**054**	Swine influenza virus dynamics in sow herds over time	**A. Diaz**, M. Torremorell; Veterinary Population Medicine, University of Minnesota, Saint Paul, MN, USA.
2:00	**055**	Antimicrobial susceptibilities of *Escherichia coli* isolated from feces of swine fed with chlortetracycline or copper	**G.E. Agga**[1], H.M. Scott[1], J. Vinasco-Torres[1], R.G. Amachawadi[1], T.G. Nagaraja[1], M. Tokach[2], J. Nelssen[2], S. Dritz[1], D.G. Renter[1], J. Bai[1], B. Norby[3]; [1]Department of Diagnostic Medicine/Pathobiology, Kansas State University, Manhattan, KS, USA, [2]Department of Animal Sciences, Kansas State University, Manhattan, KS, USA, [3]Department of Large Animal Clinical Sciences, Michigan State University, East Lansing, MI, USA.
2:15	**056**	Role of environment in the persistence of antimicrobial resistant Salmonella in antimicrobial free (ABF) and conventional pigs at farm and slaughter	**S. Keelara**[1], W.A. Gebreyes[2], W.M. Morrow[3], H.M. Scott[4], M. Correa[1], S. Thakur[1]; [1]Dept. of Population Health and Pathobiology, North Carolina State University, Raleigh, NC, USA, [2]Dept. of Veterinary Preventive Medicine, The Ohio State University, Columbus, OH, USA, [3]Dept. of Animal Science, North Carolina State University, Raleigh, NC, USA, [4]Dept. of Diagnostic Medicine/Pathobiology, Kansas State University, Manhattan, KS, USA.
2:30	**057**	Risk factors for environmental contamination with *Salmonella enterica* in a veterinary teaching hospital	**B.A. Burgess**, P.S. Morley; Clinical Sciences, Colorado State University, Fort Collins, CO, USA.
2:45		**Break and Table Top Exhibits – Foyer**	
3:00 Mon.	**058**	Perceptions of veterinarians and producers concerning Johne's disease in US beef cow-calf operations	**B. Bhattarai**[1], G.T. Fosgate[2], J.B. Osterstock[3], C.P. Fossler[4], S.C. Park[5], A.J. Roussel[6]; [1]Veterinary Integrative Biosciences, Texas A&M University, College Station, TX, USA, [2]Department of Production Animals Studies, University of Pretoria, Onderstepoort, South Africa, [3]Pfizer Animal Health, Kalamazoo, MI, USA, [4]National Animal Health Monitoring System, USDA:APHIS:VS:CEAH, Ft. Collins, CO, USA, [5]Texas AgriLife Research and Extension Center, Vernon, TX, USA, [6]Department of Large Animal Clinical Sciences, Texas A&M University, College Station, TX, USA.

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EPIDEMIOLOGY AND ANIMAL HEALTH ECONOMICS
Salons A/B/C/D - 5th Floor
Section Leader: Ashley Hill

Time	No.	Title	Authors
3:15 Mon.	**059**	Effect of individual animal calving pens on peripartum transmission of Mycobacterium avium subsp. paratuberculosis in Holstein heifer calves.	**P. Pithua**[1], L. Espejo[2], S.M. Godden[2], S.J. Wells[2]; [1]Department of Veterinary Medicine and Surgery, University of Missouri, Columbia, MO, USA, [2]Department of Veterinary Population Medicine, University of Minnesota, St. Paul, MN, USA
3:30	**060**	Effect of delayed exposure of dairy cattle to Mycobacterium avium subsp. paratuberculosis on age at first test positive and clinical Johne's disease	L. Espejo, N. Kubat, S. Godden, **S. Wells**; Veterinary Population Medicine, University of Minnesota, St. Paul, MN, USA.
3:45	**061**	Does colostrum intake affect the development of the rectal microbiota in pre-weaning dairy calves?	**L. Tomassini**[1], J.K. Harris[2], W.M. Sischo[3]; [1]Department of Veterinary Clinical Science, Washington State University, Pullman, WA, USA, [2]Department of Pediatrics, Pulmonary Medicine, School of Medicine, University of Colorado, Aurora, CO, USA, [3]Veterinary Clinical Science, Washington State University, Pullman, WA, USA
4:00 Mon.	**062**	Metagenomic versus microbiological culture based approaches to evaluate the effects of interventions strategies on ceftiofur and tetracycline resistance in cattle feces	**N. Kanwar**[1], H.M. Scott[1], B. Norby[2], G.H. Loneragan[3], J. Vinasco[1], J.L. Cottell[4], G. Chalmers[4], M.M. Chengappa[1], J. Bai[1], P. Boerlin[4]; [1]Diagnostic Medicine/ Pathobiology, Kansas State University, Manhattan, KS, USA, [2]Large Animal Clinical Sciences, Michigan State University, East Lansing, MI, USA, [3]Animal and Food Sciences, Texas Tech University, Lubbock, TX, USA, [4]Pathobiology, University of Guelph, Guelph, ON, Canada.
4:15 Mon.	**063**	Asymptomatic endemic *Chlamydia pecorum* infections reduce growth rates in calves by up to 48 percent	**A. Poudel**[1], T.H. Elsasser[2], K.S. Rahman[1], E.U. Chowdhury[1], B. Kaltenboeck[1]; [1]Pathobiology, Auburn University, Auburn, AL, USA, [2]Bovine Functional Genomics Laboratory, USDA - Agricultural Research Service, Beltsville, MD, USA.
4:30 to	**5:00**	**Break and Table Top Exhibits – Foyer**	
5:00 to	**6:30**	**Poster Session II Grand Ballroom Salon III - 7th floor**	

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EPIDEMIOLOGY AND ANIMAL HEALTH ECONOMICS
Salons A/B/C/D - 5th Floor
Section Leader: Ashley Hill

Time	No.	Title	Authors
8:00-8:45 Tues. Keynote	**031**	**Companion Animal Epidemiology Keynote in Salons A/B/C/D Rm, 5th Floor:** From licking stamps to clicking buttons - moving from conventional questionnaires to online surveys	**M.G. Doherr**; Dept. Clin. Res. & Vet. Public Health, Vetsuisse Faculty, University of Bern, Bern-Liebefeld, Switzerland.
8:45	**032**	**Mark Gearhart Memorial Graduate Student Award presented in Salons A/B/C/D Rm, 5th Floor:** Nasal shedding of Equine Herpesvirus-1 from horses in an outbreak of Equine Herpes Myeloencephalopathy in Western Canada	**B.A. Burgess**1, N. Tokateloff2, K. Poirier2, S. Manning2, K. Lohmann2, D.P. Lunn3, S.B. Hussey3, P.S. Morley3; 1University of Saskatchewan, Saskatoon, Canada; and, Animal Population Health Institute, Colorado State University, Fort Collins, CO, USA, 2Large Animal Clinical Sciences, Western College of Veterinary Medicine, University of Saskatchewan, Saskatoon, SK, Canada, 3Animal Population Health Institute, Colorado State University, Fort Collins, CO, USA.
9:00	**064**	Livestock Deaths in Mangarabombang Subdistrict, Takalar District, South Sulawesi Province, Indonesia, 2011-2012: Application of Epidemiological Investigation	**D.K. Nugroho**[1], .. Pudjiatmoko[1], M. Sybli[1], B. Poermadjaja[1], M.R. Ghani[2], S. Tum[3], K. Chanachai[4], L. Schoonman[5]; [1]Directorate of Animal Health, Ministry of Agriculture, Jakarta, Indonesia, [2]Office of Agriculture and Forestry, Takalar District, Indonesia, [3]Food and Agriculture Organization of the United Nation, Regional Office for Asia and the Pacific, Bangkok, Thailand, [4]Field Epidemiology Training Program for Veterinarians, Bangkok, Thailand, [5]Food and Agriculture Organization of the United Nation, Jakarta, Indonesia.
9:15	**065**	Open	
9:30		**Break and Table Top Exhibits – Foyer**	
10:00 Tues.	**066**	Using quarterly earnings to assess return to function in Thoroughbred racehorses after either modified laryngoplasty or colic surgery	**H. Aceto**, L.S. Southwood, S.K. Hart, E.J. Parente; Clinical Studies - New Bolton Center, University of Pennsylvania School of Veterinary Medicine, Kennett Square, PA, USA.
10:15 Tues.	**067**	Minimization of bovine tuberculosis control costs in US cattle herds	**R.L. Smith**[1], L.W. Tauer[2], Z. Lu[1], Y.H. Schukken[3], Y.T. Grohn[1]; [1]Population Medicine and Diagnostic Sciences, Cornell University College of Veterinary Medicine, Ithaca, NY, USA, [2]Dyson School of Applied Economics and Management, Cornell University, Ithaca, NY, USA, [3]Quality Milk Production Services, Cornell University College of Veterinary Medicine, Ithaca, NY, USA.

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EPIDEMIOLOGY AND ANIMAL HEALTH ECONOMICS
Salons A/B/C/D - 5th Floor
Section Leader: Ashley Hill

Time	No.	Title	Authors
10:30 Tues.	068	Patterns of cattle farm visitation by white-tailed deer in relation to bovine tuberculosis transmission risk in Minnesota	**J. Ribeiro Lima**[1], M. Carstensen[2], L. Cornicelli[2], J.D. Forester[3], M. Grund[2], S.J. Wells[1]; [1]Veterinary Population Medicine, University of Minnesota, St. Paul, MN, USA, [2]Minnesota Department of Natural Resources, St. Paul, MN, USA, [3]Department of Fisheries, Wildlife, and Conservation Biology, University of Minnesota, St. Paul, MN, USA.
10:45	069	A comparison of real and synthetic population datasets for simulation modeling of highly pathogenic avian influenza (H5N1) in commercial poultry flocks in South Carolina.	**A. Reeves**[1], M.K. Martin[2], K.A. Patyk[3], J. Helm[2], T.J. Keefe[4], B.A. Wagner[3], M.D. Salman[1], A.E. Hill[5]; [1]Department of Clinical Sciences, Colorado State University, Fort Collins, CO, USA, [2]Livestock Poultry Health Division, Clemson University, Columbia, SC, USA, [3]USDA-APHIS-VS-CEAH, Fort Collins, CO, USA, [4]Department of Environmental Health & Radiological Health Sciences, Colorado State University, Fort Collins, CO, USA, [5]California Animal Health and Food Safety Laboratory System Thurman Laboratory, University of California-Davis, Davis, CA, USA.
11:00	070	Density and distribution of backyard poultry flocks in metropolitan Denver, Colorado	**K.J. Cadmus**[1], R.S. Miller[2], M. Farnsworth[2], K.E. Slota[3], S.M. Millonig[1], K. Forde-Folle[2], R.A. Bowen[1], K.L. Pabilonia[1]; [1]College of Veterinary Medicine and Biomedical Sciences, Colorado State University, Fort Collins, CO, USA, [2]Centers for Epidemiology and Animal Health, Animal and Plant Health Inspection Service, United States Department of Agriculture, Fort Collins, CO, USA, [3]Formerly of College of Veterinary Medicine and Biomedical Sciences, Colorado State University, Fort Collins, CO, USA
11:15 Tues.	071	Antimicrobial resistance in fecal E.coli of Holstein calves housed individually or in group pens.	**R.V. Pereira**, L.D. Lorin, J.D. Siler; College of Veterinary Medicine - Population Medicine and Diagnostic Sciences (VTPMD), Cornell University, Ithaca, NY, USA.
11:45 to	12:30	**Business Meeting, Dedication, New Members Introduction, and Graduate Student Competition Awards Presentations**	

FOOD AND ENVIRONMENTAL SAFETY
Salon E - 5th Floor
Section Leader: Yvette Johnson-Walker

Time	No.	Title	Authors
		Presiders: Guy Loneragan	
8:00 Mon.	**072**	Herd prevalence and geographic distribution of *Coxiella burnetii* in cattle bulk tank milk samples in Indiana	**A.E. Bauer**[1], A.J. Johnson[1], M. Cooper[2]; [1]Comparative Pathobiology, Purdue University, Lafayette, IN, USA, [2]Indiana State Board of Animal Health, Indianapolis, IN, USA.
8:15	**073**	Prevalence, distribution, and diversity of Salmonella subtypes on Michigan dairy farms in 2000-2001 and 2009.	**G.G. Habing**[1], C. Bolin[2], S. Manning[3], J.B. Kaneene[1]; [1]Center for Comparative Epidemiology, Michigan State University, East Lansing, MI, USA, [2]Diagnostic Center for Population and Animal Health, Michigan State University, East Lansing, MI, USA, [3]Microbial and Molecular Genetics, Michigan State University, East Lansing, MI, USA.
8:30	**074**	Salmonella enterica in lymph nodes of cull and fed cattle at harvest	**H.E. Webb**[1], G.H. Loneragan[1], S.E. Gragg[1], M.M. Brashears[1], K.K. Nightingale[1], T.M. Arthur[2], J.M. Bosilevac[2], N. Kalchayanand[2], J.W. Schmidt[2], R. Wang[2], D.M. Brichta-Harhay[2]; [1]Department of Animal and Food Sciences, Texas Tech University, Lubbock, TX, USA, [2]U.S. Meat Animal Research Center, USDA-ARS, Clay Center, NE, USA.
8:45	**075**	*Salmonella* recovery from the peripheral lymph nodes following intradermal administration and evaluation of a commercially-available *Salmonella* vaccine	**T. Edrington**[1], G. Loneragan[2], J. Hill[1], K. Genovese[1], H. He[1], T. Callaway[1], R. Anderson[1], D. Brichta-Harhay[3], D. Nisbet[1]; [1]Food and Feed Safety Research Unit, USDA-ARS, College Station, TX, USA, [2]Department of Animal and Food Sciences, Texas Tech University, Lubbock, TX, USA, [3]Roman L. Hruska U.S. Meat Animal Research Center, USDA-ARS, Clay Center, NE, USA.
9:00	**076**	Sub-optimal thermal environment is associated with Salmonella shedding in swine.	**A.F.A. Pires**[1], J. Funk[1], R. Manuzon[2], L. Zhao[2]; [1]Large Animal Clinical Sciences, MSU, East Lansing, MI, USA, [2]Department of Food, Agricultural and Biological Engineering, OSU, Columbus, OH, USA.
9:15	**077**	A mathematical model to quantify effectiveness of cleaning as a measure to control Salmonella Typhimurium on a grower-finisher pig farm	**R. Gautam**, R. Ivanek; Integrative Veterinary Biosciences, Texas A&M, College Station, TX, USA.
9:30		**Break and Table Top Exhibits – Foyer**	
		Presiders: Renata Ivanek and Barbara Szonyi	
10:00 Mon.	**078**	False attribution: the effects of bias in probabilistic source attribution models for Salmonella infection	**M.R. Mason**[1], R.S. Singer[2]; [1]School of Public Health, University of Minnesota, Saint Paul, MN, USA, [2]School of Veterinary Medicine, University of Minnesota, Saint Paul, MN, USA.

FOOD AND ENVIRONMENTAL SAFETY
Salon E - 5th Floor
Section Leader: Yvette Johnson-Walker

Time	No.	Title	Authors
10:15 Mon.	079	The role of flagella in the attachment of *Salmonella* enterica serovar Kentucky to broiler skin.	**S. Salehi**[1], A. Karsi[2], M.L. Lawrence[2], J.P. Brooks[3], R.H. Bailey[1]; [1]Pathobiology and Population Medicine, Mississippi State University, Mississippi State, MS, USA, [2]Department of Basic Science, Mississippi State University, Mississippi State, MS, USA, [3]Genetics and Precision Agriculture, USDA-ARS, Mississippi State, MS, USA
10:30	080	CTX-M-type extended spectrum β-lactamase genes in *Salmonella* spp. from livestock clinical diagnostic submissions in the US	**D.F. Mollenkopf**[1], T.E. Wittum[1], M.M. Erdman[2]; [1]Veterinary Preventive Medicine, The Ohio State University, Columbus, OH, USA, [2]USDA, APHIS, VS, NVSL, Ames, IA, USA.
10:45	081	Molecular characterization of the monophasic and non-motile variants of *Salmonella enterica* serotype Typhimurium	**M. Bugarel**[1], M.-L. Vignaud[2], P. Fach[2], A. Brisabois[2]; [1]Department of Animal and Food Sciences, Texas Tech University, Lubbock, TX, USA, [2]Laboratory for Food Safety, French Agency for Food, Environmental and Occupational Health & Health (ANSES), Paris, France
11:00	082	Multi-level analysis of *Campylobacter* flock status at post-chill and risk factors within the grow-out environment	**K.L. Hataway**[1], R.H. Bailey[1], J.A. Byrd[2], V.V. Volkova[3], R.W. Wills[1]; [1]Pathobiology and Population Medicine, Mississippi State College of Veterinary Medicine, Starkville, MS, USA, [2]USDA ARS, SPARC, College Station, TX, USA, [3]Cornell University, Ithica, NY, USA.
11:15	083	Serotype distribution and antimicrobial resistance profiles of Salmonella, E. coli, and Campylobacter isolates obtained from three broiler production systems in Ontario	T.E. Roberts[1], **M.T. Guerin**[1], R. Reid-Smith[2], S.A. McEwen[1], J.M. Sargeant[3], A. Agunos[2], D. Léger[2]; [1]Population Medicine, University of Guelph, Guelph, ON, Canada, [2]Laboratory for Foodborne Zoonoses, Public Health Agency of Canada, Guelph, ON, Canada, [3]Center for Foodborne Public Health and Zoonoses, University of Guelph, Guelph, ON, Canada
11:30		**Lunch Break**	
		Presiders: Yvette Johnson-Walker	
1:30 Mon.	084	Prevalence and fluorquinolone-susceptibilities of Campylobacter and Salmonella in cattle feces from feedlots that use fluoroquinolone therapy for bovine respiratory disease	**A.B. Smith**, D.G. Renter, X. Shi, T.G. Nagaraja; Diagnostic Medicine/Pathobiology, Kansas State University, Manhattan, KS, USA.
1:45	085	Temporal changes in antimicrobial resistance within Michigan dairy cows	**E.M. Corbett**[1], B. Norby[1], L.W. Halbert[1], J.B. Kaneene[2], D.L. Grooms[1]; [1]Large Animal Clinical Sciencs, Michigan State University, East Lansing, MI, USA, [2]Center for Comparative Epidemiology, Michigan State University, East Lansing, MI, USA

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FOOD AND ENVIRONMENTAL SAFETY
Salon E - 5th Floor
Section Leader: Yvette Johnson-Walker

Time	No.	Title	Authors
2:00 Mon.	**086**	Prevalence of pathogenic shiga toxin producing Escherichia coli in dairy cattle and wildlife in Texas	**M. Subbiah**; Veterinary Integrative Biosciences, Texas A & M University, College Station, TX, USA.
2:15	**087**	Epidemiology of Shiga toxin-producing Escherichia coli (STEC) shedding in finishing swine- a descriptive longitudinal study	**M. Tseng**[1], P. Fratamico[2], L. Bagi[2], D. Manzinger[2], B. Garman[2], J. Funk[1]; [1]Michigan State University, College of Veterinary Medicine, East Lansing, MI, USA, [2]United States Department of Agriculture, Agricultural Research Service, Eastern Regional Research Center, Wyndmoor, PA, USA.
2:30	**088**	*Escherichia coli* O104 is prevalent in feces of feedlot cattle, but isolated strains did not carry genes characteristic of enterohemorrhagic or enteroaggregative pathotype	Z.D. Paddock, J. Bai, X. Shi, **T.G. Nagaraja**; Department of Diagnostic Medicine and Pathobiology, Kansas State University, Manhattan, KS, USA.
2:45		**Break and Table Top Exhibits – Foyer**	
		Presiders: Amy Baur	
3:00 Mon.	**089**	Antibiotic use versus antibiotic resistance profiles of commensal *E. coli* in beef cattle: explaining their association via bacterial growth parameters	**M. McGowan**[1], H.M. Scott[1], N. Kanwar[1], J.L. Cottell[2], P. Boerlin[2], B. Norby[3], G.H. Loneragan[4]; [1]Diagnostic Medicine and Pathobiology, Kansas State University, Manhattan, KS, USA, [2]Department of Pathobiology, University of Guelph, Guelph, ON, Canada, [3]Department of Large Animal Clinical Sciences, Michigan State University, East Lansing, MI, USA, [4]Department of Animal and Food Sciences, Texas Tech University, Lubbock, TX, USA.
3:15	**090**	Commercial evaluation of an SPR-containing *Escherichia coli* bacterial extract vaccine	**R.M. McCarthy**[1], G.H. Loneragan[1], H. Donely[2], L.I. Wright[3], D.U. Thomson[4], J.B. Morgan[5], K.K. Nightingale[1], M.M. Brashears[1]; [1]Animal and Food Sciences, Texas Tech University, Lubbock, TX, USA, [2]Beef Marketing Group, Manhattan, KS, USA, [3]3Tyson Foods, Dakota Dunes, SD, USA, [4]Kansas State University, Manhattan, KS, USA, [5]Pfizer Animal Health, Madison, NJ, USA.
3:30	**091**	Evaluation of plasmid stability in green fluorescent protein-labeled Escherichia coli O157 in a non-selective, nutrient deficient environment	**A.K. Persad**, M.L. Williams, J.T. LeJeune; Food Animal Health Research Program, The Ohio State University, Wooster, OH, USA.
3:45 Mon.	**092**	Effect of flavophospholipol and environment on antimicrobial resistance in beef cattle.	**S.A. Ison**[1], G.H. Loneragan[1], S.T. Trojan[1], M.M. Brashears[1], B. Norby[2], H.M. Scott[3]; [1]Animal and Food Science, Texas Tech University, Lubbock, TX, USA, [2]Michigan State University, East Lansing, MI, USA, [3]Kansas State University, Manhattan, KS, USA.

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FOOD AND ENVIRONMENTAL SAFETY
Salon E - 5th Floor
Section Leader: Yvette Johnson-Walker

Time	No.	Title	Authors
4:00 Mon.	**093**	Discovery of novel alternatives to antibiotic growth promoter to protect food safety	Z. Wang, X. Zeng, Y. Mo, **K. Smith**, J. Lin; Animal Science, University of Tennessee, Knoxville, TN, USA.
4:15	**094**	Prevalence of transferable copper resistance gene, *tcrB* , in fecal enterococci of feedlot cattle fed diets supplemented with copper	**R.G. Amachawadi**[1], H.M. Scott[1], T.R. Mainini[1], C.A. Alvarado[2], J. Vinasco[1], T.G. Nagaraja[1], J.S. Drouillard[2]; [1]Diagnostic Medicine/Pathobiology, Kansas State University, Manhattan, KS, USA, [2]Animal Sciences and Industry, Kansas State University, Manhattan, KS, USA
4:30 to	**5:00**	**Break and Table Top Exhibits – Foyer**	
5:00 to	**6:30**	**Poster Session II Grand Ballroom Salon III - 7th floor**	

FOOD AND ENVIRONMENTAL SAFETY
Salon E - 5th Floor
Section Leader: Yvette Johnson-Walker

Time	No.	Title	Authors
		Presiders: Maung San Myint	
8:00-8:45 Tues. Keynote	**031**	**Companion Animal Epidemiology Keynote in Salons A/B/C/D Rm, 5th Floor:** From licking stamps to clicking buttons - moving from conventional questionnaires to online surveys	**M.G. Doherr**; Dept. Clin. Res. & Vet. Public Health, Vetsuisse Faculty, University of Bern, Bern-Liebefeld, Switzerland.
8:45	**032**	**Mark Gearhart Memorial Graduate Student Award in Salons A/B/C/D Rm, 5th Floor: :** Nasal shedding of Equine Herpesvirus-1 from horses in an outbreak of Equine Herpes Myeloencephalopathy in Western Canada	**B.A. Burgess**1, N. Tokateloff2, K. Poirier2, S. Manning2, K. Lohmann2, D.P. Lunn3, S.B. Hussey3, P.S. Morley3; 1University of Saskatchewan, Saskatoon, Canada; and, Animal Population Health Institute, Colorado State University, Fort Collins, CO, USA, 2Large Animal Clinical Sciences, Western College of Veterinary Medicine, University of Saskatchewan, Saskatoon, SK, Canada, 3Animal Population Health Institute, Colorado State University, Fort Collins, CO, USA.
9:00	**095**	An agent-based model to assess the potential effects of vaccines in *Escherichia coli* O157 shedding and transmission in feedlots	S. Chen[1], M. Sanderson[2], **C. Lanzas**[1]; [1]Biomedical and Diagnostic Sciences, University of Tennessee, Knoxville, TN, USA, [2]Diagnostic Medicine and Pathobiology, Kansas State University, Manhattan, KS, USA.
9:15 Tues.	**096**	The impact of vaccination and post-harvest intervention failures on beef carcass contamination with E. coli O157	**M. Jacob**[1], M. Sanderson[2], C. Dodd[3], D. Renter[2]; [1]North Carolina State University, Raleigh, NC, USA, [2]Kansas State University, Manhattan, KS, USA, [3]248th Medical Detachment, US Army Veterinary Corps, Ft. Bragg, NC, USA
9:30		**Break and Table Top Exhibits – Foyer**	
		Presiders: Yvette Johnson-Walker	
10:00 Tues.	**097**	Development of a loop-mediated isothermal amplification assay for point-of-need detection of *Escherichia coli*	**J. Chandler**, L. Goodridge; Colorado State University, Fort Collins, CO, USA.
10:15 Tues.	**098**	Evaluating on-farm interventions to reduce antimicrobial resistance in enteric commensal *Escherichia coli* of cattle with mathematical modeling	**V. Volkova**[1], Z. Lu[1], C. Lanzas[2], Y. Grohn[1]; [1]Department of Population Medicine and Diagnostic Sciences, College of Veterinary Medicine, Cornell University, Ithaca, NY, USA, [2]Department of Biomedical and Diagnostic Sciences, College of Veterinary Medicine, University of Tennessee, Knoxville, TN, USA.
10:30	**099**	Impact of feeding distillers grain-based diets on the colonic microbial community structure of cattle	S. Park[1], **M. Williams**[2], J. LeJeune[2], S. Loerch[3], B. McSpadden Gardener[1]; [1]Plant Pathology, OARDC/ Ohio State University, Wooster, OH, USA, [2]Food Animal Health Research Program, OARDC/ Ohio State University, Wooster, OH, USA, [3]Animal Science, OARDC/ Ohio State University, Wooster, OH, USA.

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FOOD AND ENVIRONMENTAL SAFETY
Salon E - 5th Floor
Section Leader: Yvette Johnson-Walker

Time	No.	Title	Authors
		Presiders: Yvette Johnson-Walker	
10:45 Tues.	**100**	An analysis of foodborne illness risk factor violations and bacterial load in restaurant food preparation areas.	**M. Myint**[1], Y.J. Walker[1], V. Eisenbart[1], P. Liles[2]; [1]Veterinary Clinical Medicine, UIUC, College of Veterinary Medicine, Urbana, IL, USA, [2]Environmental Health, Champaign-Urbana Public Health District, Champaign, IL, USA
11:00	**101**	*In vitro* effect of deoxynivalenol (DON) mycotoxin on porcine circovirus type 2 (PCV2) replication and cytopathic effect.	**C. Savard**, C. Provost, V. Pinilla, M. Segura, C.A. Gagnon, Y. Chorfi; Faculté de médecine Vétérinaire, Université de montréal, Saint-Hyacinthe, QC, Canada.
11:15	**102**	A one health approach to public health issues in Ghana	**Y.J. Johnson**; Center for One Health Illinois, University of Illinois, Urbana-Champaign, Urbana, IL, USA.
11:45 to	**12:30**	**Business Meeting, Dedication, New Members Introduction, and Graduate Student Competition Awards Presentations**	

GASTROENTERIC DISEASES
Michigan/Michigan State Room - 6th Floor
Section Leaders: Radhey S. Kaushik and David H. Francis

Time	No.	Title	Authors
		Presiders: Radhey S. Kaushik and David H. Francis	
8:00 to	**8:45**	Open	
8:45-9:30 Mon. Keynote	**103**	**Keynote**: Unveiling the mysteries of iron regulation in *Mycobacterium avium* subspecies *paratuberculosis*	**S. Sreevatsan**; Veterinary Population Medicine and Veterinary Biomedical Sciences Departments, CVM, University of Minnesota, St. Paul, MN, USA.
9:30		**Break and Table Top Exhibits – Foyer**	
10:00	**104**	A novel vaccine candidate protecting cattle against diarrhea caused by enterotoxigenic Escherichia coli (ETEC) and bovine viral diarrhea virus (BVDV)	**E.A. Hashish**[1], D.E. Knudsen[1], C.C.L. Chase[1], R. Isaacson[2], W. Zhang[1]; [1]Veterinary and biomedical science department, South Dakota State University, Brookings, SD, USA, [2]Department of Veterinary and Biomedical Sciences, College of Veterinary Medicine, University of Minnesota, St. Paul, MN, USA.
10:15	**105**	A genetic fusion of enterotoxins of enterotoxigenic Escherichia coli (ETEC) induced broadly antitoxin immunity against ETEC associated diarrhea	**D.J. Rausch**, C. Zhang, X. Ruan, E. Hashish, W. Zhang; Department of Veterinary and Biomedical Sciences, South Dakota State University, Brookings, SD, USA.
10:30	**106**	Safety and immunogenicity studies of a modified heat-labile toxin (LT) and heat-stable toxin (ST) fusion protein (LTS63K/R192G/L211A-3xSTaA14Q) in a murine model	**C. Zhang**[1], M. Liu[1], D. Knudsen[1], S. Lawson[1], D. Robertson[2], W. Zhang[1]; [1]Veterinary and Biomedical Sciences, South Dakota State University, Brookings, SD, USA, [2]Diagnostic Medicine/Pathobiology, Kansas State University, Manhattan, KS, USA.
10:45 Mon.	**107**	Development of a modified live vaccine against enterotoxigenic *Escherichia coli*-associated porcine post-weaning diarrhea	**X. Ruan**, C. Zhang, W. Zhang; Veterinary & Biomedical Science, South Dakota State University, Brookings, SD, USA.
11:00	**108**	Glucose significantly affects enterotoxigenic Escherichia coli adherence to intestinal epithelial cells through its effects on heat-labile enterotoxin production	**P. Wijemanne**, R. Moxley; School of Veterinary Medicine and Biomedical Sciences, University of Nebraska-Lincoln, Lincoln, NE, USA.
11:15	**109**	Laser capture microdissection coupled with RNA-seq analysis to evaluate the transcriptional response of pigs experimentally infected with Lawsonia intracellularis	**F.A. Vannucci**[1], D. Foster[2], C. Gebhart[1]; [1]College of Veterinary Medicine, University of Minnesota, St. Paul, MN, USA, [2]College of Food, Agricultural and Natural Resource Science, University of Minnesota, St. Paul, MN, USA
11:30		**Lunch Break**	
		Presiders: Radhey S. Kaushik and David H. Francis	
1:30 Mon.	**110**	Development of novel vaccines for mitigation of Campylobacter in poultry	**L. Jones**, X. Zeng, J. Lin; Animal Science, The University of Tennessee, Knoxville, TN, USA.
1:45	**111**	Comparative pathogenicity of porcine rotavirus group A, B and C in neonatal pigs	**H.T. Hoang**, D. Madson, P. Arruda, G. Stevenson, K.-J. Yoon; Veterinary Diagnostics & Production Animal Medicine, Iowa State University, Ames, IA, USA.

GASTROENTERIC DISEASES
Michigan/Michigan State Room - 6th Floor
Section Leaders: Radhey S. Kaushik and David H. Francis

Time	No.	Title	Authors
		Presiders: Radhey S. Kaushik and David H. Francis	
2:00 Mon.	**112**	Characterization of porcine group B rotavirus G genotype in the United States reveals substantial genetic diversity	**D. Marthaler**[1], K. Rossow[1], M. Gramer[1], J. Collins[1], S. Goyal[1], H. Tsunemitsu[2], K. Kuga[2], T. Suzuki[2], M. Ciarlet[3], J. Matthijnssens[4]; [1]University of Minnesota, St. Paul, MN, USA, [2]National Institute of Animal Health, Ibaraki, Japan, [3]Novartis Vaccines and Diagnostics, Cambrige, MA, USA, [4]Department of Microbiology and Immunology, Laboratory of Clinical and Epidemiological Virology, Leuven, Belgium
2:15	**113**	Characterization of swine group C rotavirus G genotypes from the United States and Canada reveals a new proposed G genotype	**D. Marthaler**[1], K. Rossow[1], M. Marie[1], J. Collins[1], S. Goyal[1], M. Ciarlet[2], J. Matthijnsen[3]; [1]University of Minnesota, St. Paul, MN, USA, [2]Novartis Vaccines and Diagnostics, Cambridge, MA, USA, [3]Department of Microbiology and Immunology, Laboratory of Clinical and Epidemiological Virology, Leuven, Belgium
2:30 Mon.	**114**	The effect of climate change on the evolution of food- and waterborne diseases: a systematic review.	**K. Henn**, S. Ilic, J. LeJeune; Ohio Agricultural Research and Development Center, OSU, Wooster, OH, USA.
2:45		**Break and Table Top Exhibits – Foyer**	
4:30 to	**5:00**	**Break and Table Top Exhibits – Foyer**	
5:00 to	**6:30**	**Poster Session II Grand Ballroom Salon III - 7th floor**	

IMMUNOLOGY
Salons F/G/H - 5th Floor
Section Leader: Laura C. Miller

Time	No.	Title	AuthorBlock
		Mini-Symposium	
		Presiders: Susan Eicher and Laura Miller	
8:00-8:30 Mon.	**115**	Bovine tuberculosis research: Immune mechanisms relevant to biomedical applications	**R. Waters**[1], M. Palmer[1], J. Telfer[2], C. Baldwin[3]; [1]Tuberculosis Research Project, National Animal Disease Center, Ames, IA, USA, [2]University of Massachusetts, Amherst, MA, USA, [3]University of Massachusetts, Amherst, IA, USA
8:30	**116**	The role of bovine γδ T cells and their WC1 co-receptor in interacting with bacterial pathogens and promoting vaccine efficacy.	**C.L. Baldwin**[1], C. Chen[1], H. Hsu[1], R. Waters[2], M. Palmer[2], J. Telfer[1]; [1]Veterinary and Animal Sciences, University of Massachusetts, Amherst, MA, USA, [2]Infectious Bacterial Diseases of Livestock Research, USDA National Animal Disease Center, Ames, IA, USA
8:45	**117**	Characterization of the antigen-specific γδ T cell response following virulent Mycobacterium bovis infection in cattle	**J.L. McGill**[1], J.C. Telfer[2], C.L. Baldwin[2], R.E. Sacco[1], M.V. Palmer[3], W.R. Waters[3]; [1]Ruminant Diseases and Immunology Research Unit, National Animal Disease Center, Agricultural Research Service, United States Department of Agriculture, Ames, IA, USA, [2]Department of Veterinary and Animal Sciences, University of Massachusetts, Amherst, MA, USA, [3]Infectious Bacterial Diseases Research Unit, National Animal Disease Center, Agricultural Research Service, United States Department of Agriculture, Ames, IA, USA.
9:00	**118**	WC1 functions as a pattern recognition receptor and co-receptor for γδ T cells	**H.-T. Hsu**, C. Chen, C.L. Baldwin, J.C. Telfer; Department of Veterinary & Animal Sciences, UMass Amherst, Amherst, MA, USA.
9:15	**119**	Effector and memory T cell subsets in the response to bovine tuberculosis.	**M.F. Maggioli**[1], M.V. Palmer[1], H.M. Vordermeier[2], D.M. Estes[3], W.R. Waters[1]; [1]1Infectious Bacterial Diseases of Livestock Research Unit, National Animal Disease Center, Ames, IA, USA, [2]TB Research Group, Animal Health and Veterinary Laboratories Agency-Weybridge, New Haw, Addlestone, UK, [3]Department of Infectious Disease, University of Georgia, Athens, GA, USA.
9:30		**Break and Table Top Exhibits – Foyer**	
		Presiders: Carol Chitko-McKown and Lorraine Sordillo	
10:00-10:30 Mon.	**120**	Preterm piglets are a clinically relevant model of pediatric GI disease	**D. Burrin**[1], N. Ghoneim[1], B. Stoll[1], T. Thymann[2], P. Sangild[2]; [1]Department of Pediatrics, USDA-Children's Nutrition Research Center, Houston, TX, USA, [2]Department of Human Nutrition, University of Copenhagen, Copenhagen, Denmark

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IMMUNOLOGY
Salons F/G/H - 5th Floor
Section Leader: Laura C. Miller

Time	No.	Title	AuthorBlock
		Presiders: Carol Chitko-McKown and Lorraine Sordillo	
10:30-11:00	**121**	The Pig as a Model for the Study of Adipose Tissue Dysfunction in Obesity	**K. Ajuwon**, Department of Animal Sciences, Purdue University, West Lafayette, IN, USA.
11:00 Mon.	**122**	Nanoparticle based inactivated adjuvanted porcine reproductive and respiratory syndrome virus vaccine elicits superior cross protective immunity	**B. Binjawadagi**[1], V. Dwivedi[1], C. Manickam[1], K. Ouyang[1], J.B. Torrelles[2], R. Gourapura[1]; [1]FAHRP-OARDC (Department of Veterinary Preventive Medicine), The Ohio State University, Wooster, OH, USA, [2]Department of Microbial Infection and Immunity, The Ohio State University, Columbus, OH, USA.
11:15	**123**	H9e peptide hydrogel: a novel adjuvant for PRRS modified live virus vaccine	**X. Li**[1], A. Galliher-Beckley[1], J. Nietfeld[2], H. Huang[3], S. Sun[3], K. Faaberg[4], J. Shi[1]; [1]Anatomy and Physiology, Kansas State University, Manhattan, KS, USA, [2]Diagnostic Medicine, Kansas State University, Manhattan, KS, USA, [3]Grain Science, Kansas State University, Manhattan, KS, USA, [4]NADC, Ames, IA, USA
11:30		**Lunch Break**	
		Presiders: Isis Mullarky and Glenn Zhang	
1:30-2:15 Mon. Keynote	**124**	**Keynote-Distinguished Veterinary Immunologist:** Moving Swine Immunology Forward Through Molecular and Vaccine Technology.	**Michael P. Murtaugh**, Veterinary and Biomedical Sciences, CVM, University of Minnesota, St. Paul, MN.
2:15	**125**	Development of a mouse model for delineating protective immune response(s) specific for epizootic bovine abortion	**R. Brooks**, M. Blanchard, J. Stott; UC Davis, Davis, CA, USA.
2:30	**126**	Use of dermal fibroblasts to predict the innate immune response to bovine mastitis	**A.L. Benjamin**, D.E. Kerr; Animal Science, University of Vermont, Burlington, VT, USA.
2:45		**Break and Table Top Exhibits – Foyer**	
		Presiders: Katherine Petersson and Bill Davis	
3:00	**127**	The potential contribution of epigenetic modifications to the animal-specific responses of dermal fibroblasts to LPS.	**B.B. Green**, S.D. McKay, D.E. Kerr; University of Vermont, Burlington, VT, USA.
3:15	**128**	Interleukin-8 receptor expression in bovine mammary tissue.	**L. Siebert**[1], J. Lippolis[2], G.M. Pighetti[1]; [1]The University of Tennessee, Knoxville, TN, USA, [2]USDA-NADC, Ames, IA, USA.
3:30	**129**	Lipid metabolism by bovine mammary endothelial cells during *Streptococcus uberis* mastitis	**V.E. Ryman**, C.M. Corl, L.M. Sordillo; Large Animal Clinical Sciences, Michigan State University, East Lansing, MI, USA.
3:45 Mon.	**130**	Increased linoleic acid in post-partum bovine monocytes is associated with proinflammatory phenotype.	**W. Raphael**, L.M. Sordillo; Large Animal Clinical Sciences, Michigan State University, East Lansing, MI, USA.

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IMMUNOLOGY
Salons F/G/H - 5th Floor
Section Leader: Laura C. Miller

Time	No.	Title	AuthorBlock
		Presiders: Katherine Petersson and Bill Davis	
4:00 Mon.	**131**	Age-related susceptibility to R equi infection in foals	**L. Sun**[1], M.G. Sanz[1], A.T. Loynachan[2], A. Page[1], D.W. Horohov[1]; [1]Veterinary Science, Gluck Equine Research Center, Lexington, KY, USA, [2]Veterinary Science, 2Veterinary Diagnostic Laboratory, Lexington, KY, USA
4:15 to	**4:30**	Open	
4:30 to	**5:00**	**Break and Table Top Exhibits – Foyer**	
5:00 to	**6:30**	**Poster Session II Grand Ballroom Salon III - 7th floor**	

IMMUNOLOGY
Salons F/G/H - 5th Floor
Section Leader: Laura C. Miller

Time	No.	Title	Authors
		Presiders: Laurel Gershwin and Renukaradhya Gourapura	
8:00 to	**8:15**	Open	
8:15 Tues.	**132**	Evaluation of a live attentuated vaccine for Johne's disease	**W.C. Davis**, K. Park, A.J. Allen, G.M. Barrington; Vet. Micro/Pathol, Wash State Univ., Pullman, WA, USA.
8:30	**133**	Tumor necrosis factor (TNF)-α diminishes the ability of bovine macrophage to cleave extracellular traps formed in response to *M. haemolytica*	**N.A. Aulik**[1], K.M. Hellenbrand[2], D.N. Atapattu[2], C.J. Czuprynski[3]; [1]Department of Pathobiological Sciences, School of Vet Med., University of Wisconsin-Madison; and, Winona State University, Biology Department, Winona, MN, USA, [2]Department of Pathobiological Sciences, School of Veterinary Medicine, University of Wisconsin-Madison, Madison, WI, USA, [3]Food Research Institute; and the, Department of Pathobiological Sciences, School of Vet Med., University of Wisconsin-Madison, Madison, WI, USA.
8:45	**134**	Induction of osteopontin expression in bovine mammary endothelial cells	**C.M. Corl**, L.M. Sordillo; Large Animal Clinical Sciences, Michigan State University, East Lansing, MI, USA.
9:00	**135**	Genetic variation in CXCR1 amino acid expression significantly tied to clearance of Streptococcus uberis in an intramammary challenge model	**G.M. Pighetti**[1], S. Headrick[1], M. Lewis[2], B. Gillespie[1], C. Young[2], L. Siebert[1], L. Wojakiewicz[1], O. Kerro Dego[1], R. Almeida[1], S.P. Oliver[3]; [1]Department of Animal Science, University of Tennessee, Knoxville, TN, USA, [2]East Tennessee Research and Education Center, University of Tennessee, Knoxville, TN, USA, [3]AgResearch, University of Tennessee, Knoxville, TN, USA
9:15		Open	
9:30 Tues.		**Break and Table Top Exhibits – Foyer**	
11:45 to	**12:30**	**Business Meeting, Dedication, New Members Introduction, and Graduate Student Competition Awards Presentations**	

RESPIRATORY DISEASES
Indiana/Iowa Room 6th Floor
Section Leaders: Amelia Woolums and Christopher Chase

Time	No.	Title	Authors
		Presiders: Chris Chase and Amelia Woolums	
8:00 Mon.	**137**	Comparing Influenza A virus isolation from oral fluid and nasal swabs in IAV inoculated pigs	**C.K. Goodell**, F. Zhou, C. Wang, K.-J. Yoon, R. Main, J. Zimmerman; Veterinary Diagnostic and Production Animal Medine, Iowa State University College of Veterinary Medicine, Ames, IA, USA.
8:15	**138**	Comparing Influenza A virus detection in oral fluid and nasal swabs by a rapid antigen detection kit in IAV inoculated pigs	**C.K. Goodell**[1], A. Kittawornrat[1], Y. Panyasing[1], C. Olsen[1], T. Overbay[2], C. Wang[1], R. Main[1], J. Zimmerman[1]; [1]Veterinary Diagnostic and Production Animal Medine, Iowa State University College of Veterinary Medicine, Ames, IA, USA, [2]Abaxis, Inc, Union City, CA, USA
8:30	**139**	Comparing Influenza A virus detection in oral fluid and nasal swabs by RT-PCR in IAV inoculated pigs	**C.K. Goodell**[1], R. Rauh[2], W. Nelson[2], C. O'Connell[3], A. Burrell[3], C. Wang[1], R. Main[1], J. Zimmerman[1]; [1]Veterinary Diagnostic and Production Animal Medine, Iowa State University College of Veterinary Medicine, Ames, IA, USA, [2]Tetracore, Inc, Rockville, MD, USA, [3]Life Technologies Corporation, Carlsbad, CA, USA
8:45	**140**	Kinetics of influenza A virus (IAV) anti-nucleoprotein antibody (IgM, IgA, IgG) in serum and oral fluid specimens	**Y. Panyasing**[1], C. Goodell[1], L. Giménez-Lirola[1], A. Kittawornrat[1], C. Wang[1], S. Lizano[2], A. Ballagi[2], P. Lopez[2], K. Schwartz[1], J. Zimmerman[1]; [1]Veterinary Diagnostic and Production Animal Medicine, Iowa State University, Ames, IA, USA, [2]IDEXX laboratories, Inc., westbrook, ME, USA.
9:00	**141**	Natural killer T cell specific adjuvants potentiates cell-mediated immunity in the pig lungs to an inactivated bivalent swine influenza H1N1 and H3N2 virus vaccine	**C. Manickam**, K. Ouyang, B. Binjawadagi, P. Crittenden, J. Hiremath; Food Animal Health Research Program, The Ohio State University, wooster, OH, USA.
9:15	**142**	Immortalized swine bone marrow epithelial cell line supports influenza virus replication.	**M. Khatri**; Food Animal Health, Ohio State University, Wooster, OH, USA.
9:30		**Break and Table Top Exhibits – Foyer**	
10:00 Mon.	**143**	Priming by respiratory exposure followed by intramascular boost with RNA particle vaccine in pigs in an influenza challenge model	**Q. Chen**[1], D. Madson[2], C. Miller[1], D. Harris[1]; [1]VMPM, Iowa State University, Ames, IA, USA, [2]VDPAM, Iowa State University, Ames, IA, USA

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RESPIRATORY DISEASES
Indiana/Iowa Room 6th Floor
Section Leaders: Amelia Woolums and Christopher Chase

Time	No.	Title	Authors
		Presiders: Chris Chase and Amelia Woolums	
10:15 Mon.	**144**	A novel DNA vaccine provided efficient protection to mice against lethal dose of swine influenza virus H1N1	H. Wei[1], S. Lenz[2], D. Thompson[3], **R.M. Pogranichniy**[2]; [1]Comparative Pathobiology, Purdue University, W. Lafayette, IN, USA, [2]Comparative Pathobiology, and Animal Disease Diagnostic Laboratory, Purdue University, W. Lafayette, IN, USA, [3]Chemistry, Purdue University, W. Lafayette, IN, USA.
10:30	**145**	Migration of the swine influenza virus delta-cluster hemagglutinin N-linked glycosylation site from N142 to N144 results in loss of antibody cross reactivity	**B. Hause**[1], D. Stine[1], Z. Sheng[2], Z. Wang[2], S. Chakravarty[2], R. Simonson[1], F. Li[2]; [1]Newport Labs, Worthington, MN, USA, [2]South Dakota State University, Brookings, SD, USA.
10:45	**146**	Inactivation of Swine Influenza Virus with imidazolidinyl urea with retention of hemagglutination activity	**M. Inman**, L. Trygstad, M.A. Pfannenstiel; Research and Development, Benchmark Biolabs, Lincoln, NE, USA.
11:00	**147**	Full genome of swine influenza (H1N1) in pigs using next generation sequencing	**A. Diaz**, S. Enomoto, S. Sreevatsan, M. Gramer, M. Torremorell; Veterinary Population Medicine, University of Minnesota, Saint Paul, MN, USA.
11:15	**148**	Genome evolution and antigenic variation of canine influenza virus H3N8 in U.S. dogs	**H.L. Pecoraro**, S. Bennett, M. Spindel, G. Landolt; Clinical Sciences, Colorado State University, Fort Collins, CO, USA.
11:30		**Lunch Break**	
		Presiders: Chris Chase and Amelia Woolums	
1:30 to	**1:45**	Open	
1:45 Mon.	**149**	Zoonotic tuberculosis in pastoralists and their livestock in Ethiopia	**B.G. Donde**[1], E. Schelling[2], A. Aseffa[3], J. Zinsstag[2]; [1]Animal Science, Bule Hora University, Bule Hora, Ethiopia, [2]Epidemiology and Public Health, Swiss Tropical and Public Health Institute, Basel, Switzerland, [3]Armauer Hansen Research Institute, Addis Ababa, Ethiopia
2:00	**150**	The impact of gastrointestinal nematode parasitism on the response of calves to viral respiratory vaccination	**A. Woolums**[1], R. Berghaus[2], R. Kaplan[3], D. Hurley[2], R. Ellis[2], J. Saliki[4], L. Berghaus[1], S. Howell[3], M. Thoresen[1], D. Major[1], C. Reyner[1]; [1]Large Animal Medicine, CVM, University of Georgia, Athens, GA, USA, [2]Population Health, CVM, University of Georgia, Athens, GA, USA, [3]Infectious Diseases, CVM, University of Georgia, Athens, GA, USA, [4]Athens Veterinary Diagnostic Laboratory, CVM, University of Georgia, Athens, GA, USA.
2:15 Mon.		Open	

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RESPIRATORY DISEASES
Indiana/Iowa Room 6th Floor
Section Leaders: Amelia Woolums and Christopher Chase

Time	No.	Title	Authors
		Presiders: Chris Chase and Amelia Woolums	
2:30 Mon.	**152**	In vitro and in vivo prostaglandin E2 synthesis in BRSV infection and modulation by COX inhibition	**L.J. Gershwin**[1], R. Toaff-Rosenstein[2], M. Shao[1], H. McEligot[1], L. Corbeil[3], C. Tucker[2]; [1]Pathology, Microbioloby, & Immunology, University of California, Davis, Davis, CA, USA, [2]Animal Science, University of California, Davis, Davis, CA, USA, [3]Pathology, School of Medicine UCSD, and Population Health, UCD, University of California, San Diego and Davis, San Diego and Davis, CA, USA.
2:45		**Break and Table Top Exhibits – Foyer**	
3:00	**153**	Prevalence of viral and bacterial pathogens in nasopharyngeal and pharyngeal recess regions of Holstein calves with and without signs of clinical bovine respiratory disease	**T.W. Lehenbauer**[1], S.S. Aly[1], J.H. Davis[1], P.C. Blanchard[2], B.M. Crossley[3], P.V. Rossitto[1], H.L. Neibergs[4], A.L. Van Eenennaam[5]; [1]Veterinary Medicine Teaching and Research Center, University of California Davis, Tulare, CA, USA, [2]California Animal Health & Food Safety Laboratory System, University of California Davis, Tulare, CA, USA, [3]Medicine and Epidemiology, University of California Davis, Davis, CA, USA, [4]Animal Science, Washington State University, Pullman, WA, USA, [5]Animal Science, University of California Davis, Davis, CA, USA.
3:15	**154**	Pathogenicity of *Bibersteinia trehalosi* in cattle	**C.J. Hanthorn**, G.A. Dewell, V.L. Cooper, R.D. Dewell, J.J. Ninneman, P.J. Plummer; Veterinary Diagnostic and Production Animal Medicine, Iowa State University, Ames, IA, USA.
3:30	**155**	RNA-Seq based structural re-annotation of BRD bacterial pathogens	J.S. Reddy[1], S.C. Burgess[2], B. Nanduri[1], **M.L. Lawrence**[1]; [1]College of Veterinary Medicine, Mississippi State University, Mississippi State, MS, USA, [2]College Agriculture and Life Sciences, University of Arizona, Tuscon, AZ, USA
3:45-4:30 Mon. Keynote	**156**	**Keynote:** *Mannheimia haemolytica* immunity. Are we there yet?	**A.W. Confer**; Veterinary Pathobiology, Oklahoma State University, Stillwater, OK, USA.
4:30 to	**5:00**	**Break and Table Top Exhibits – Foyer**	
5:00 to	**6:30**	**Poster Session II Grand Ballroom Salon III - 7th floor**	

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RESPIRATORY DISEASES
Indiana/Iowa Room 6th Floor
Section Leaders: Amelia Woolums and Christopher Chase

Time	No.	Title	Authors
		PRRSV Mini-Symposium	
		Presiders: Kay Faaberg and Apisit Kittawornrat	
8:00 to	**8:15**	Open	
8:15 Tues.	**157**	DNA shuffling of the GP3 genes of PRRSV produces a chimeric virus with an improved cross-neutralizing ability against a heterologous PRRSV strain	**L. Zhou**, Y.-Y. Ni, P. Piñeyro, B.J. Sanford, C.M. Cossaboom, B.A. Dryman, Y.-W. Huang, D.-J. Cao, X.-J. Meng; Virginia Tech, blacksburg, VA, USA.
8:30	**158**	Characterization of the neutralizing antibody response to PRRSV	J. Li, J.C. Schwartz, S.R. Robinson, **M.P. Murtaugh**; Veterinary and Biomedical Sciences, University of Minnesota, St. Paul, MN, USA.
8:45	**159**	Development of swine oral fluid based porcine reproductive and respiratory syndrome virus neutralizing assay: a potential diagnostic tool for PRRS herd immunity	B. Binjawadagi[1], **K. Ouyang**[1], N. Elkalifa[1], J. Wu[2], C. Olsen[3], J. Zimmerman[3], R.J. Gourapura[1]; [1]FAHRP, OARDC,The Ohio State University, wooster, OH, USA, [2]Vererinary Research Institute of Guangxi, Nanning, China, [3]Veterinary Diagnostic and Production Animal Medicine, Iowa State University, Ames, IA, USA.
9:00 Tues.	**160**	Effect of sample collection material on the detection of PRRSV in oral fluid	**C. Olsen**[1], J. Coetzee[1], L. Karriker[1], A. Kittawornrat[1], S. Lizano[2], R. Main[1], A. Meiszberg[1], Y. Panyasing[1], C. Wang[3], J. Zimmerman[1]; [1]Veterinary Diagnostic and Production Animal Medicine, Iowa State University, Ames, IA, USA, [2]IDEXX Laboratories Inc., Westbrooke, ME, USA, [3]Veterinary Diagnostic and Production Animal Medicine and Department of Statistics, Iowa State University, Ames, IA, USA.
9:15 Tues.	**161**	Probability of detecting PRRSV infection using pen-based swine oral fluid specimens as a function of within-pen prevalence	**C. Olsen**[1], C. Wang[2], J. Christopher-Hennings[3], K. Doolittle[4], A. Kittawornrat[1], A. Kurtz[5], E. Kurtz[5], S. Lizano[6], R. Main[1], T. Otterson[7], Y. Panyasing[1], C. Rademacher[5], R. Rauh[8], R. Shah[9], J. Zimmerman[1]; [1]Veterinary Diagnostic and Production Animal Medicine, Iowa State University, Ames, IA, USA, [2]Veterinary Diagnostic and Production Animal Medicine and Department of Statistics, Iowa State University, Ames, IA, USA, [3]Veterinary and Biomedical Sciences, South Dakota State University, Brookings, SD, USA, [4]Health Management Center, Behringer Ingelheim Vetmedica, Inc., Ames, IA, USA, [5]Western Operations, Murphy-Brown LLC, Ames, IA, USA, [6]IDEXX Laboratories Inc., Westbrooke, ME, USA, [7]Veterinary Diagnostic Laboratory, University of Minnesota, Saint Paul, MN, USA, [8]Tetracore Inc., Rockville, MD, USA, [9]Animal, Food and Environmental Testing Group, Life Technologies, Austin, TX, USA
9:30		**Break and Table Top Exhibits – Foyer**	

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RESPIRATORY DISEASES
Indiana/Iowa Room 6th Floor
Section Leaders: Amelia Woolums and Christopher Chase

Time	No.	Title	Authors
		PRRSV Mini-Symposium	
		Presiders: Kay Faaberg and Apisit Kittawornrat	
10:00 Tues.	**162**	Detection of PRRSV antibody in oral fluid specimens from individual boars using a commercial prrsv serum antibody elisa.	**A. Kittawornrat**[1], M. Engle[2], Y. Panyasing[1], C. Olsen[1], K. Schwartz[1], S. Lizano[3], C. Wang[1], J. Zimmerman[1]; [1]Veterinary Diagnostic and Production Animal Medicine, Iowa State University, ames, IA, USA, [2]PIC North America, Hendersonville, TN, USA, [3]IDEXX Laboratories, Westbrook, ME, USA
10:15 Tues.	**163**	Ring test evaluation for the detection of PRRSV antibody in oral fluid specimens using a commercial PRRSV serum antibody ELISA.	**A. Kittawornrat**[1], C. Wang[1], G. Anderson[2], A. Ballagi[3], A. Broes[4], S. Carman[5], K. Doolittle[6], J. Galeota[7], J. Johnson[1], S. Lizano[3], E. Nelson[8], D. Patnayak[9], R. Pogranichniy[10], A. Rice[3], G. Scherba[11], J. Zimmerman[1]; [1]Veterinary Diagnostic and Production Animal Medicine, Iowa State University, ames, IA, USA, [2]Veterinary Diagnostic Laboratory, Kansas State University, Manhattan, KS, USA, [3]IDEXX Laboratories, Westbrook, ME, USA, [4]Biovet Inc., Saint-Hyacinthe, QC, Canada, [5]University of Guelph, Guelph, ON, Canada, [6]Boehringer Ingelheim Vetmedica Inc., St. Joseph, MO, USA, [7]University of Nebraska, Lincoln, NE, USA, [8]South Dakota State University, Brookings, SD, USA, [9]University of Minnesota, St Paul, MN, USA, [10]Purdue University, West Lafayette, IN, USA, [11]
10:30	**164**	The antiviral activity of *Actinobacillus pleuropneumoniae* against Porcine reproductive and respiratory syndrome virus in the porcine alveolar macrophages	**Y. Hernandez Reyes**, C. Provost, J. Ferreira-Barbosa, J. Labrie, C. Gagnon, M. Jacques; Faculty of Medicin Veterinary, Université de Montréal, Saint-Hyacinthe, QC, Canada.
10:45-11:30 Tues.	**202**	**Viral Pathogenesis Keynote in Los Angeles - Miami Room, 5th Floor:** Of Men, Pigs, Birds and…Flu.	**Daniel R. Perez,** Veterinary Medicine, University of Maryland, College Park, MD
11:45 to	**12:30**	**Business Meeting, Dedication, New Members Introduction, and Graduate Student Competition Awards Presentations**	

VECTOR-BORNE AND PARASITIC DISEASES
Denver/Houston - 5th Floor
Section Leader: Roman Ganta

Time	No.	Title	Authors
		Presiders: Roman Ganta and Roger Stich	
8:00 Mon.	**165**	Targeted and Random Mutagenesis of *Ehrlichia chaffeensis* for the Identification of Genes Required for *In vivo* Infection	C. Cheng[1], A.D.S. Nair[1], V.V. Indukuri[1], S. Gong[1], R.F. Felsheim[2], D. Jaworski[3], U.G. Munderloh[2], **R. Ganta**[1]; [1]Department of Diagnostic Medicine/Pathobiology, College of Veterinary Medicine, Kansas State University, Manhattan, KS, USA, [2]Department of Entomology, University of Minnesota, St. Paul, MN, USA, [3]Department of Entomology and Plant Pathology, Oklahoma State University, Noble Research Center, Stillwater, OK, USA.
8:15	**166**	Exploratory spatial data analysis of human Lyme disease cases in Texas between 2000 and 2010	**B. Szonyi**, I. Srinath, M. Esteve-Gassent, B. Lupiani, R. Ivanek; Texas A&M University, College Station, TX, USA.
8:30	**167**	Transplacental transmission of a human isolate of *Anaplasma phagocytophilum* in an experimentally infected sheep.	**E.J. Reppert**[1], R.C. Galindo[2], M.A. Breshears[2], K.M. Kocan[2], E.F. Blouin[2], J. de la Fuente[3]; [1]Veterinary Clinical Sciences, Center for Veterinary Health Sciences Oklahoma State University, Stillwater, OK, USA, [2]Veterinary Pathobiology, Center for Veterinary Health Sciences Oklahoma State University, Stillwater, OK, USA, [3]Instituto de Investigacion en Recursos Cinegeticos IREC (CSIC-USLM-JCCM), Ciudad Real, Spain.
8:45	**168**	Inactivation of bacteria in milk using a flow-through UV-light treatment system.	**R.V. Pereira**, M.L. Bicalho, V.S. Machado, S. Lima, A.G. Teixeira, R.C. Bicalho; College of Veterinary Medicine - Population Medicine and Diagnostic Sciences (VTPMD), Cornell University, Ithaca, NY, USA.
9:00	**169**	Temporal and spatial distribution of borreliosis, ehrlichiosis, anaplasmosis, and Rocky Mountain spotted fever in humans and dogs in Illinois from 2000-2009.	**N.M. Dahm**, J.A. Herrmann; University of Illinois College of Veterinary Medicine, Urbana-Champaign, IL, USA.
9:15	**170**	Evaluation of the systemic inflammatory reaction to anthelmintic treatment in ponies	**A. Betancourt**, J.C. Stewart, E.T. Lyons, D.W. Horohov, M.K. Nielsen; Veterinary Science, University of Kentucky, Lexington, KY, USA.
9:30		**Break and Table Top Exhibits – Foyer**	
10:00-10:45 Mon. Keynote	**171**	**Keynote:** Recent advances in research of the Q fever bacterium, Coxiella burnetii	**R. Heinzen**; Coxiella Pathogenesis Section, National Institute for Allergy and Infectious Disease, National Institutes of Health, Hamlton, MT, USA.

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VECTOR-BORNE AND PARASITIC DISEASES
Denver/Houston - 5th Floor
Section Leader: Roman Ganta

Time	No.	Title	Authors
		Presiders: Roman Ganta and Roger Stich	
10:45 Mon.	**172**	The ecology of eastern equine encephalitis virus in wildlife and mosquitoes in Minnesota	**A.C. Kinsley**[1], E. Butler[2], R. Moon[3], K. Johnson[4], M. Carstensen[2], D. Neitzel[5], M.E. Craft[1]; [1]Veterinary Population Medicine, University of Minnesota, St. Paul, MN, USA, [2]Minnesota Department of Natural Resources, Forest Lake, MN, USA, [3]Entomology, University of Minnesota, St. Paul, MN, USA, [4]Metropolitan Mosquito Control District, St. Paul, MN, USA, [5]Minnesota Department of Health, St. Paul, MN, USA.
11:00	**173**	Anthelmintic effect of proanthocyanidin extract of cranberry leaf powder on *Haemonchus contortus* and *Caenorhabiditis elegans*	A. Zajac[1], L. Manzi[2], L. Katiki[3], A. Giudice[1], **K. Petersson**[2]; [1]Biomedical Sciences and Pathobiology, VA-MD Regional College of Veterinary Medicine, Virginia Tech, Blacksburg, VA, USA, [2]Fisheries, Animal & Veterinary Science, University of Rhode Island, Kingston, RI, USA, [3]Instituto de Zootecnia (SAA-APTA), Nova Odessa-Sao Paulo, Brazil.
11:15 Mon.	**174**	The chlamydiosis pathogenesis studies at experimental infection of white rats	**V. Skrypnyk**, I. Ksyonz, A. Skrypnyk; SSCIBMS, Kyiv, Ukraine.
11:30		**Lunch Break**	
4:30 to	**5:00**	**Break and Table Top Exhibits – Foyer**	
5:00 to	**6:30**	**Poster Session II Grand Ballroom Salon III - 7th floor**	

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

Time	No.	Title	Authors
		PRRSV Mini-Symposium	
8:00 Mon.	**175**	A novel small structural protein ORF5a is essential for porcine reproductive and respiratory syndrome virus production	**B. Kwon**, H.L.X. Vu, L.K. Beura, S. Subramaniam, A.K. Pattnaik, F.A. Osorio; Nebraska Center for Virology and School of Veterinary Medicine and Biomedical Sciences, University of Nebraska-Lincoln, Lincoln, NE, USA.
8:15	**176**	Virion packaging of multiple cleavage isoforms of porcine reproductive and respiratory syndrome virus nonstructural protein 2	**M.A. Kappes**, K.S. Faaberg; Virus and Prion Research Unit, USDA-ARS-National Animal Disease Center, Ames, IA, USA.
8:30	**177**	Host cell gene expressions and cell cycle progression regulated by PRRS virus Nsp11 protein	**D. Yoo**[1], Y. Sun[1], D. Li[1], S. Giri[2], S.G. Prasanth[2]; [1]Department of Pathobiology, University of Illinois at Urbana-Champaign, Urbana, IL, USA, [2]Department of Cell and Developmental Biology, University of Illinois at Urbana-Champaign, Urbana, IL, USA.
8:45	**178**	Suppression of host gene expression by nsp1β protein of porcine reproductive and respiratory syndrome virus	Y. Li, S. Lawson, Z. Sun, **Y. Fang**; Department of Veterinary and Biomedical Sciences; Department of Biology/Microbiology, South Dakota State University, Brookings, SD, USA.
9:00	**179**	The PRRSV-mediated inhibition of interferon alpha production by its natural host cell occurs at the post-transcriptional level.	**W.-Y. Chen**, G. Calzada-Nova, W. Schnitzlein, F.A. Zuckermann; Department of Pathobiology, University of Illinois, Urbana-Champaign, IL, USA.
9:15	**180**	Variable interference with interferon signal transduction by different PRRSV strains	R. Wang, Y. Nan, Y. Yu, **Y. Zhang**; Molecular Virology Laboratory, VA-MD Regional College of Veterinary Medicine, University of Maryland, College Park, MD, USA.
9:30		**Break and Table Top Exhibits – Foyer**	
10:00 Mon.	**181**	Identification of regulatory domain of PRRS virus nonstructural protein 1 alpha for type I interferon modulation	**M. Han**, Y. Du, C. Song, D. Yoo; Department of Pathobiology, University of Illinois at Urbana-Champaign, Urbana, IL, USA.
10:15	**182**	PRRSV nsp1β inhibits interferon signal transduction by inducing importin-α5 degradation	**R. Wang**, Y. Nan, Y. Yu, Y. Zhang; Molecular Virology Laboratory, VA-MD Regional College of Veterinary Medicine, University of Maryland, College Park, MD, USA.
10:30	**183**	The disease manifestations of two Asian highly pathogenic strains of Type 2 PRRSV	**K.S. Faaberg**[1], K.M. Lager[1], B. Guo[2], S.L. Brockmeier[1], L.C. Miller[1], J.N. Henningson[1], S.N. Schlink[1], M.A. Kappes[1], M.E. Kehrli, Jr[1], T.L. Nicholson[1], S.L. Swenson[3], H.-C. Yang[4]; [1]Virus and Prion Research Unit, USDA-ARS-NADC, Ames, IA, USA, [2]Veterinary Diagnostic & Production Animal Medicine, Iowa State University, Ames, IA, USA, [3]Virology, USDA-APHIS-NVSL, Ames, IA, USA, [4]China Agricultural University, Beijing, China.

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

Time	No.	Title	Authors
		PRRSV Mini-Symposium	
10:45 Mon.	**184**	Comparison of Asian highly-pathogenic PRRSV isolates to US isolates for their ability to cause secondary bacterial infection in swine	**S.L. Brockmeier**, C.L. Loving, M.V. Palmer, A.R. Spear, K.S. Faaberg, T.L. Nicholson; Virus and Prion Research Unit, National Animal Disease Center, Ames, IA, USA.
11:00	**185**	Changes in circulating and thymic lymphocyte populations following infection with strains of North American or Highly Pathogenic PRRSV.	**C.L. Loving**[1], S. Brockmeier[1], M. Palmer[2], A. Spear[2], K. Faaberg[2], T. Nicholson[1]; [1]Respiratory Diseases of Swine, USDA-ARS-National Animal Disease Center, Ames, IA, USA, [2]USDA-ARS-National Animal Disease Center, Ames, IA, USA
11:15	**186**	Swine tracheobronchial lymph node mRNA responses in swine infected with a highly pathogenic strain of Porcine Reproductive and Respiratory Syndrome virus.	**L.C. Miller**[1], D. Fleming[2], A. Arbogast[3], D.O. Bayles[4], B. Guo[5], K.M. Lager[1], J.N. Henningson[1], S.N. Schlink[1], H.-C. Yang[6], K.S. Faaberg[1], M.E. Kehrli, Jr.[1]; [1]Virus and Prion Diseases Research Unit, USDA-ARS-National Animal Disease Center, Ames, IA, USA, [2]Inter-departmental Genetics, Iowa State University, Ames, IA, USA, [3]Department of Computer Science, Iowa State University, Ames, IA, USA, [4]Infectious Bacterial Diseases Research Unit, USDA-ARS-National Animal Disease Center, Ames, IA, USA, [5]Veterinary Diagnostic & Production Animal Medicine, Iowa State University, Ames, IA, USA, [6]China Agricultural University, Beijing, China.
11:30		**Lunch Break**	
		PRRSV Mini-Symposium	
1:30 Mon.	**187**	Attenuation of porcine reproductive and respiratory syndrome virus by molecular breeding of the virus envelope genes from genetically divergent strains	**Y.-Y. Ni**[1], T. Opriessnig[2], L. Zhou[1], D. Cao[1], Y.-W. Huang[1], P.G. Halbur[2], X.-J. Meng[1]; [1]Department of Biomedical Sciences and Pathobiology, Virginia Polytechnic Institute and State University, Blacksburg, VA, USA, [2]Department of Diagnostic and Animal Production Medicine, Iowa State University, Ames, IA, USA
1:45	**188**	Development of a modified live vaccine against porcine reproductive and respiratory syndrome with optimal “DIVA” marker potential	**H. Vu**[1], B. Kwon[1], M. de Lima[2], A. Pattnaik[1], F. Osorio[1]; [1]Veterinary medicine and Biomedical Sciences, University of Nebraska-Lincoln, Lincoln, NE, USA, [2]Faculdade de Veterinaria, Universidade Federal de Pelotas, Pelotas, Brazil
2:00	**189**	Flexible polymer adjuvants for live and inactivated vaccines: Application to PRRS live vaccine	R. Parker[1], **J. Ben Arous**[2], S. Deville[2], F. Bertrand[2], L. Dupuis[2]; [1]SEPPIC Inc, Fairfield, NJ, USA, [2]SEPPIC, Puteaux, France

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

Time	No.	Title	Authors
		PRRSV Mini-Symposium	
2:15 Mon.	**190**	Novel simian hemorrhagic fever viruses from wild African primates offer new insights into the evolutionary origins of PRRSV	**T.L. Goldberg**[1], D.H. O'Connor[2], T. Friedrich[2], M. Lauck[2], S. Sibley[2], D. Hyeroba[3], A. Tumukunde[3], G. Weny[3], J.H. Kuhn[4]; [1]Pathobiological Sciences, University of Wisconsin-Madison, Madison, WI, USA, [2]University of Wisconsin-Madison, Madison, WI, USA, [3]Makerere University, Kampala, Uganda, [4]Pathobiological Sciences, g. Integrated Research Facility at Fort Detrick, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Fort Detrick,
2:30	**191**	Validation of an equine arteritis virus antibody cELISA according to OIE protocol.	**C. Chung**[1], C. Wilson[1], E. Adams[1], D.S. Adams[1], J. Evermann[2], P. Timoney[3], A. Clavijo[4], S. Rogers[4], S.S. Lee[5], T.C. McGuire[1]; [1]R&D, VMRD Inc., Pullman, WA, USA, [2]WADDL, Pullman, WA, USA, [3]University of Kentucky, Lexington, KY, USA, [4]TVMDL, College Station, TX, USA, [5]Department of Statistics, University of Idaho, Moscow, ID,
2:45		**Break and Table Top Exhibits – Foyer**	
3:00 Mon.	**192**	Isolation of a novel swine influenza virus distantly related to influenza C	**B. Hause**[1], M. Ducatez[2], E. Collin[1], A. Armien[3], B. Kaplan[2], R. Webby[2], R. Simonson[1], F. Li[4]; [1]Newport Labs, Worthington, MN, USA, [2]St. Jude Children's Research Hospital, Memphis, TN, USA, [3]University of Minnesota, St. Paul, MN, USA, [4]South Dakota State University, Brookings, SD, USA.
3:15	**193**	Harnessing RNAi to inhibit avian influenza replication in avian cells using a novel delivery technology: Progressing towards an alternative prevention strategy.	**L.M. Linke**[1], J. Fruehauf[2], G. Landolt[3], R. Magnuson[1], J. Wilusz[4], M. Salman[1]; [1]Clinical Sciences: Animal Population Health Institute, Colorado State University, Fort Collins, CO, USA, [2]Cambridge Biolabs, Cambridge, MA, USA, [3]Clinical Sciences, Colorado State University, Fort Collins, CO, USA, [4]Microbiology, Immunology and Pathology, Colorado State University, Fort Collins, CO,
3:30 Mon.	**194**	Pathogenicity and transmissibility of novel reassortant H3N2 swine influenza viruses with the 2009 pandemic H1N1 genes in pigs	J. Ma[1], H. Shen[1], Q. Liu[1], B. Bawa[1], J. Richt[1], R. Hesse[1], S. Henry[2], **W. Ma**[1]; [1]Diagnostic Medicine/Pathobiology, Kansas State University, Manhattan, KS, USA, [2]Abilene Animal Hospital PA, Abilene, KS, USA.

VIRAL PATHOGENESIS
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Time	No.	Title	Authors
3:45 Mon.	195	Development of an equine ocular endothelial cell model to study equine herpesvirus myelitis (EHM)	**G.S. Hussey**[1], L.S. Goehring[2], D.P. Lunn[3], S.B. Hussey[2], C. Powell[2], J. Hand[2], K. Osterrieder[4], J. Slater[5]; [1]Pathobiology and Diagnostic Investigation, Michigan State University, East Lansing, MI, USA, [2]Clinical Sciences, Colorado State University, Fort Collins, CO, USA, [3]North Carolina State University, Raleigh, NC, USA, [4]Freihe Universitaet Berlin, Berlin, Germany, [5]Royal Veterinary College, Hatfield, UK.
4:00 to	**4:30**	Open	
4:30 to	**5:00**	**Break and Table Top Exhibits – Foyer**	
5:00 to	**6:30**	**Poster Session II Grand Ballroom Salon III - 7th floor**	

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

Time	No.	Title	Authors
8:00 to	**8:45**	Open	
8:45 Tues.	**196**	Group C porcine Rotavirus subunit vaccine	**K.R. Sirigireddy**, K. Wilson, T. Oleson, D. Stine, R. Simonson, R. Bey; Research and Development, Newport Laboratories, Worthington, MN, USA.
9:00	**197**	Genetic diversity of porcine circoviruses type 2 detected in pigs in Ukraine	**A.P. Gerilovych**, B.T. Stegniy, N.G. Rudova, V.I. Bolotin; Molecular epidemiology and diagnostics, NSC Institute of experimental and clinical veterinary medicine, Kharkiv, Ukraine.
9:15	**198**	Characterization of the first complete genomesequence of the North American beaver (*Castorcanadensis*) papillomavirus	**A.S. Rogovskyy**[1], R.D. Burk[2], Z. Chen[3], T. Bankhead[4]; [1]Washington Animal Disease Diagnostic Laboratory, Department of Veterinary Microbiology and Pathology, College of Veterinary Medicine, Washington State University, Pullman, WA, USA, [2]Department of Pediatrics and Department of Microbiology and Immunology, Albert Einstein College of Medicine, Bronx, NY, USA, [3]Department of Microbiology and Immunology, Albert Einstein College of Medicine, Bronx, NY, USA, [4]Department of Veterinary Microbiology and Pathology, College of Veterinary Medicine, and Paul G. Allen School for Global Animal Health, Washington State University, Pullman, WA, USA.
9:30		**Break and Table Top Exhibits – Foyer**	
10:00 Tues.	**199**	Expression of type I interferon-induced antiviral state during experimental infection with low or high virulence bovine viral diarrhea virus in beef calves	**R.A. Palomares**[1], H. Walz[2], K.V. Brock[2]; [1]Population Health, University of Georgia, Athens, GA, USA, [2]Pathobiology, Auburn University, Auburn, AL, USA
10:15 Tues.	**200**	Differential expression of pro-inflammatory and anti-inflammatory cytokines during experimental infection with low or high virulence bovine viral diarrhea virus in beef calves	**R.A. Palomares**[1], K.V. Brock[2]; [1]Population Health, University of Georgia, Athens, GA, USA, [2]Pathobiology, Auburn University, Auburn, AL, USA.
10:30	**201**	PCR-screening of chlamydia and viral contamination of bovine semen in Ukraine	**A.P. Gerilovych**, V.I. Bolotin, O.S. Solodiankin, R.O. Kucheryavenko, I.V. Goraichuk; Molecular epidemiology and diagnostics, NSC Institute of experimental and clinical veterinary medicine, Kharkiv, Ukraine.
10:45-11:30 Keynote	**202**	**Keynote**: Of Men, Pigs, Birds and…Flu.	**Daniel R. Perez**, Veterinary Medicine, University of Maryland, College Park, MD
11:45 to	**12:30**	**Business Meeting, Dedication, New Members Introduction, and Graduate Student Competition Awards Presentations**	

POSTER ABSTRACTS

BACTERIAL PATHOGENESIS POSTERS

001P

Methicillin-resistant *Staphylococcus aureus* and *Staphylococcus pseudintermedius* from companion animals and horses at a veterinary teaching hospital in Quebec, Canada.

E. Rodriguez[1], S. Messier[1], D. Daignault[2], S. Monecke[3], R. Ehricht[4], M. Archambault[1];
[1]Department of Pathology and Microbiology, Faculty of Veterinary Medicine, University of Montreal, Saint Hyacinthe, QC, Canada, [2]Canadian Integrated Program for Antimicrobial Resistance Surveillance, Health Canada, Saint Hyacinthe, QC, Canada, [3]Institute for Medical Microbiology and Hygiene, Technical University of Dresden, Dresden, Germany, [4]Alere Technologies GmbH, Jena, Germany.

Methicillin-resistant *Staphylococcus aureus* (MRSA) and *Staphylococcus pseudintermedius* (MRSP) have been recognized as significant pathogens in veterinary medicine. This study was conducted to characterize the antimicrobial resistance patterns, virulence properties and genetic relatedness of MRSA (*n*=18) and MRSP (*n*=22) isolates recovered from clinical infections in dogs, cats, and horses, their environment and one clinician (MRSA=1) at the veterinary teaching hospital of the University of Montreal. Antimicrobial resistance phenotypes were determined by broth microdilution. Virulence and resistance genes were detected by a DNAmicroarray and genomic relatedness by pulsed-field gel electrophoresis (PFGE). All isolates were susceptible to daptomycin, linezolid, quinopristin/dalfopristin and vancomycin. Rates of antimicrobial resistance for MRSA and MRSP isolates were as follows, respectively: erythromycin (94.4% and 68.2%), ciprofloxacin (66.7% and 68.2%), clindamycin (61.1% and 68.2%), gentamicin (33.3% and 63.6%), tetracyclines (27.8% and 63.6%), trimethoprim/sulfamethoxazole (33.3% and 72.7%) and rifampin (27.8 and 0%). Several antimicrobial resistance genes were detected such as *mecA, blaZ, blaI, blaR* (β-lactams), *erm*(A), *erm*(B), *erm*(C) (MLS_b), *Inu*(A) (lincosamides), *aadD, aacA-aphD,aphA3* (aminoglycosides), *dfrS1* (trimethoprim), *tet*(M), *tet*(K) (tetracyclines) and *sat* (streptothricin). The MRSA isolates were negative for Panton-Valentine leucocidin, toxic shock syndrome toxin and exfoliative toxin genes but positive for enterotoxin, haemolysin, leukocidin and superantigen genes. Two MLST-SCC*mec* clonal complexes (CC5-MRSA-II, USA100; and CC8-MRSA-IV, USA500; both human clones) and three PFGE patterns were detected in MRSA. Two SCC*mec* types (III and V) and three PFGE patterns were identified in MRSP isolates. The data demonstrate the presence of considerable resistance toward major classes of antimicrobials used in veterinary medicine indicating that these infections may represent a serious therapeutic challenge. Also, the presence of human MRSA clones in companion animals suggests possible reverse zoonosis transmission.

002P

A new drug for an old bug: Antimicrobial activity of novel substituted thiazoles against methicillin-resistant Staphylococcus aureus (MRSA)

H. Mohammad[1], A.S. Mayhoub[2], A. Ghafoor[1], M. Soofi[1], R.A. Alajlouni[1], M. Cushman[2], M.N. Seleem[1];
[1]Comparative Pathobiology, Purdue University, West Lafayette, IN, USA, [2]Medicinal Chemistry and Molecular Pharmacology, Purdue University, West Lafayette, IN, USA.

Methicillin-resistant *Staphylococcus aureus* (MRSA) is a growing global health concern due to the scarcity of effective antimicrobials. In recent years MRSA has been increasingly reported as an emerging problem in food-producing and companion animals. Whole-cell screening assays of libraries of substituted thiazole and thiadiazole derivatives revealed a novel lead antimicrobial against MRSA. A series of analogues of the lead compound, composed of a thiazole backbone connected on one end to a cationic moiety and on the other end to a lipophilic tail, were synthesized and their activity against MRSA was tested. The main alterations performed on the lead compound pertained to addition of methylene units to the alkane side chain of the lead compound and substitution of the alkane side chain with cycloalkane, cycloalkene, and arene moieties. The lead compound effectively inhibited MRSA growth at 8.0 μM. Investigation of the proper side chain length at the phenyl ring of the lipophilic tail provided the pentyl analogue and an enhanced antimicrobial activity (MIC to 3.0 μM). Moreover, the conformationally restricted biphenyl analogue revealed the most significant reduction of the MIC value (2.0 μM). A time-kill assay revealed that both the lead compound and the pentyl analogue eliminated MRSA growth over a four-hour period. However, the more potent biphenyl analogue required a longer period of time (ten hours) to completely eliminate the pathogen. *In vitro* cytotoxic analysis of the compounds against murine macrophage cells revealed four derivatives to be toxic at a concentration of 16 μM. Of the derivatives which exhibited the most potency in terms of MIC values (including the pentyl and biphenyl analogues), none were observed to be toxic. Cell leakage experiments performed demonstrated that the lead compound did not mimic the action of lysostaphin, an antibacterial enzyme capable of disrupting the cell wall of *Staphylococci* bacteria. The synthesized thiazoles show promise as a novel antimicrobial class of compounds to treat MRSA infections.

003P

Characterization of the ability of coagulase negative staphylococci isolated from milk to form biofilms.

Y.D.N. Tremblay[1], D. Lamarche[1], P. Chever[1], D. Haine[2], S. Messier[1], **M. Jacques**[1];
[1]Pathologie et Microbiologie, Université de Montréal, St-Hyacinthe, QC, Canada, [2]Sciences cliniques, Université de Montréal, St-Hyacinthe, QC, Canada.

Purpose: Mastitis is the infection of the mammary gland and, in dairy cows, it is the most common and detrimental disease which has a major economical impact on the production of milk and dairy products. Although coagulase-negative staphylococci (CNS) are considered a minor mastitis pathogen, the importance of CNS has increased over the years. The mechanism and factors involved in CNS intramammary infection (IMI) are poorly defined. Biofilms have been proposed as an important component of the persistence of CNS IMI. Biofilms are defined as a cluster of bacteria enclosed in a self-produced matrix. The objectives of this study were to investigate the ability of CNS to form biofilm. Methods: A total of 255 mastitis-associated CNS isolates was investigated using a standard microtiter plate biofilm assay. Using PCR, the presence of biofilm associated genes *icaA, bap, aap, embp, fbe* and *atlE* was determined in the 255 isolates. Results: The five species assayed were *Staphylococcus chromogenes* (n = 111), *S. simulans* (n = 53), *S. xylosus* (n = 25), *S. haemolyticus* (n = 15) and *S. epidermidis* (n = 13) and these species represented 85% of the CNS isolates. Overall, *S. xylosus* is the species with the strongest ability to form biofilm and *S. epidermidis* is the species with the lowest ability to form biofilm. Regardless of the species, the presence of *icaA, bap* or the combination of multiple genes was associated with a greater ability to form biofilm. A strong relationship between the strength of a biofilm and days in milk (DIM) was also noted and it appears that CNS isolated later in the lactation cycle have a greater ability to form a biofilm than those isolated earlier in the lactation cycle. Furthermore, confocal laser scanning microscopy analysis and enzymatic degradation of biofilms revealed the presence of protein, extracellular DNA and poly-N-acetyl-glucosamine in the matrix of the biofilm. Based on the enzymatic degradation analysis, proteins play a critical role in CSN biofilms. Conclusions: *S. xylosus* is the species with the strongest ability to form biofilm. Furthermore, DIM and gene combination are predicted to be the variables with the strongest effect on biofilm formation.

004P

Cj0843c, a putative lytic transglycosylase, is involved in beta-lactam resistance by modulating beta-lactamse activity in Campylobacter jejuni

X. Zeng, S. Brown, B. Gillespie, J. Lin; Animal Science, University of Tennessee, Knoxville, TN, USA.

Beta-lactam antibiotics are an important class of antibiotics for treating bacterial infections. Emergence of beta-lactam resistance has greatly compromised clinical effectiveness of this group of antibiotics. Despite prevalent beta-lactam resistance in Campylobacter jejuni, the leading bacteria cause of human diarrhea in USA, molecular basis of beta-lactam resistance in C. jejuni is still largely unknown. In this study, an in vivo random transposon mutagenesis

004P (continued)
was performed to identify genes required for beta-lactam resistance in C. jejuni 81-176. Screening of a 2,800-mutant library identified 22 mutants with increased susceptibility to ampicillin. Direct sequencing indicated that 20 mutants have transposons inserted in the genes encoding CmeABC drug efflux pump while other 2 have insertions in Cj0843c (a putative lytic transglycosylase gene) and its upstream gene Cj0844c. Further complementation and molecular manipulation in different strain background demonstrated that Cj0843c contributes to both intrinsic and acquired beta-lactam resistance in C. jejuni. Notably, inactivation of Cj0843c dramatically reduced beta-lactamase activity, strongly suggesting that Cj0843c modulates the expression of beta-lactamase. Genomic examination and PCR analysis also demonstrated that Cj0843c is widely distributed in C. jejuni. In summary, we have identified a novel mechanism of beta-lactam resistance in C. jejuni, which will help us better understand the development and regulation of β-lactam resistance, a significant issue in many bacterial pathogens.

005P
Inactivation of *gidB* confers low-level streptomycin resistance and compromises bacterial fitness in *Campylobacte*r
Z. Shen, L. Dai, Z. Wu, Q. Zhang; Iowa State University, Ames, IA, USA.

Campylobacter infection is the leading bacterial cause of human gastroenteritis worldwide. Usually, macrolides and fluoroquinolones are the drugs of choice for the treatment of severe campylobateriosis. However, intravenous aminoglycosides are required for serious *Campylobacter* bacteraemia. Previous studies indicated that the presence of *aadE* gene and mutations in *rpsL* are the main cause of antibiotic resistance to streptomycin (SM), one of the oldest aminoglycoside drugs, in *Campylobacter*. In this study, we evaluated the role of a putative 16S rRNA methyltransferase (*gidB*) in mediating SM resistance in *Campylobacter*. Inactivation of *gidB* in *C. jejuni* resulted in 2- and 4-fold increase in the MICs of SM and complementation of the *gidB* mutant restored the MIC of SM. Interestingly, compared with the wild type strain, the mutation frequency of SM resistance was increased by 2 to 3 log in the *gidB* mutant under 8 μg/ml SM selection pressure. Further *in vitro* competition assay showed that the *gidB* mutant was outcompeted by the wild type strain in culture media, suggesting a compromised fitness for the mutant. These results indicate that *gidB* plays a role in reducing the emergence of SM resistance and maintaining fitness in *Campylobacter*. Further studies are required to assess the contribution of *gidB* to fitness *in vivo*.

006P
Salmochelin-mediated iron acquisition in Campylobacter jejuni
Y. Mo, X. Zeng, J. Lin; Department of Animal Science, The University of Tennessee, Knoxville, TN, USA.

Campylobacter could utilize exogenous siderophores, a group of small iron chelators, to efficiently scavenge iron from environment. To date, enterobactin is the only known iron siderophore that *Campylobacter* would encounter and use in the intestine during infection. Salmochelin, a glycosylated derivative of enterobactin that could confer resistance against host innate immunity mediated by lipocalins, may be another high-affinity siderphore that could be utilized by *Campylobacter* in the intestine. To test this, in this study, salmochelins were *in vitro* synthesized by glucosylation of enterobactin using purified IroB, a *C*-glycosyltrasnferase. Salmochelin growth promotion assay indicated that *Campylobacter* could efficiently utilize salmochelin as a sole iron source for growth under iron-restricted conditions. In activation of CfrA or CfrB, the receptor for enterobactin, abolished the ability of *C. jejuni* strains to utilize salmochelin. The growth promotion assays also demonstrated that Cee, a periplasmic enterobactin esterase, also play a critical role in salmochelin-mediated iron acquisition in these strains, which is consistent with the finding that Cee could hydrolyze salmochelin *in vitro*. Together, this study firmly established that *Campylobacter* could utilize high-affinity salmochelin for iron acquisition, and provided insights into the delicate interaction between *Campylobacter* and host during infection.

007P
Research in progress: A bivalent immunocontraceptive vaccine against brucellosis in feral swine
G.P. Smith[1], P. Rajasekaran[1], S.M. Boyle[1], L.A. Miller[2], N. Sriranganathan[1]; [1]VA-MD Regional College of Veterinary Medicine, Blacksburg, VA, USA, [2]USDA National Wildlife Research Center, Fort Collins, CO, USA.

Feral swine are a nuisance species across most of the United States costing around $1bn each year in agricultural, environmental, and personal property damages. In the last ten years the population of feral swine is estimated to have quadrupled and novel population control methods are needed. Furthermore, feral swine are known carriers of multiple zoonotic diseases such as brucellosis, which threaten both livestock biosecurity and public health. Antigenic recombinant multimeric gonadotropin-releasing hormone (mGnRH) has been previously used as a subunit vaccine to induce immunocontraception in feral pigs, however multiple doses are needed to elicit a robust anti-GnRH immune response and current delivery methods are limited. It is proposed that live bacterial antigen delivery using *Brucella suis* VTRS2 as a novel platform can be employed to deliver mGnRH without the use of antibiotic resistant markers, while simultaneously conferring protection against brucellosis in feral swine. VTRS2 was created by Cre-*loxP* recombination to generate markerless deletions of the LPS biosynthesis gene *wboA* as well as the *leuB* gene required for leucine biosynthesis inside the nutrient-depleted intracellular environment occupied by *Brucella*. Mutations in *wboA* are known to attenuate *Brucella* strains such as the vaccine strain *B. abortus* RB51, however RB51 is rifampin resistant and has minimal efficacy in swine. It is hypothesized that VTRS2 will confer significantly better protection against *B. suis* challenge than RB51. Furthermore, the mGnRH antigen can be delivered using the pNS4 family of plasmids (which carry *leuB* under its native promoter) thus maintaining the plasmid under leucine-deficient conditions to confer immunocontraception in the host. These hypotheses will be tested in the murine model to determine the clearance kinetics of VTRS2 and VTRS2-mGnRH and subsequently to measure vaccine efficacy against challenge by virulent *B. suis* 1330. An improved vaccine against Brucellosis in swine, as well as one which confers immunocontraception without the use of antibiotic resistance, could become an important tool in the management of this nuisance species.

008P
Immunogenicity and safety of a natural rough mutant of *Brucella suis* as a vaccine for swine
S. Olsen[1], C.A. Johnson[1], W. Stoffregen[2]; [1]National Animal Disease Center, Ames, IA, USA, [2]Preclinical Pathology, Boston Scientific Corporation, Plymouth, MN, USA.

Purpose: The objective of the current study was to evaluate the safety, immunogenicity and clearance of the natural rough mutant of *Brucella suis* strain 353-1 (353-1) as a vaccine in domestic swine
Methods: In three studies encompassing 155 animals, pigs were inoculated with 353-1 by conjunctival (5×10^7 CFU), parenteral ($1.8\text{-}2.0 \times 10^{10}$ CFU), or oral routes (5×10^{11} CFU). Clearance, tissue distribution, and pathology of the vaccine strain were determined by periodic blood culture and analysis of tissues at necropsy. Shedding from vaccinates was determined by serologic and microbiologic evaluation of samples from co-housed sentinel animals.
Results: Strain 353-1 was non-pathogenic, stable, and cleared from most parenteral or oral vaccinates by 10 to 12 weeks after vaccination. Parenteral or oral vaccination induced significant humoral responses, peripheral blood mononuclear cell proliferation, and interferon-gamma (IFN-γ) production after inoculation when compared to responses of control pigs.

008 (continued)
Conclusions: Our data demonstrates that *B. suis* 353-1 is a stable, rough mutant that does not induce adverse clinical effects or tissue localization in vaccinated swine, but does induce significant humoral and cellular immune responses.

009P
Isolation of *Brucella* species from aborted fetuses of sheep and goats in Mongolia
K. Lee[1], M. Her[1], J.-Y. Kim[1], S.-I. Kang[1], E. Janchivdorj[2], S. Jung[1]; [1]Bacterial disease division, Animal, Plant and Fisheries Quarantine and Inspection Agency, Anyang, Korea, Republic of, [2]Immunological Research Center, Institute of Veterinary Medicine, Ulaanbaatar, Mongolia.

Purpose: Brucellosis is an important zoonotic disease that affects public health and economic losses worldwide. In Mongolia, domestic animals such as calves, sheep and goats have been immunized with attenuated live vaccine of *B. abortus* strain S-19 and *B. melitensis* strain Rev-1. In this study, we identified not only virulent strains but also vaccine strain of *Brucella* species from aborted fetuses of sheep and goats of Mongolia.
Methods: We collected gastric juices from aborted fetuses of 11 sheep and 5 goats of Mongolia in order to investigate *Brucella* infection . The samples were cultured on blood agar and modified brucella selective agar and incubated at 37℃ for four to five days under 5% CO_2. AMOS-PCR, multiplex differential PCR and real-time PCR were conducted to differentiate *Brucella* species of the isolates. The biovar of *Brucell*a species was determined by classical biotyping assay.
Results: Strains showing all the characteristics of smooth *Brucella* were isolated from all cultures. Nine out of 11 isolates from sheep were *B. melitensis* and 2 isolates were *B. abortus*. Five strains from goats were *B. melitensis*. A total of 16 isolates were *B. melitensis* by AMOS-PCR, but 2 isolates from sheep were identified as *B. abortus* by multiplex differential PCR and real-time PCR. All *B. abortus* were biovar 3. Five *B. melitensis* isolates from sheep were biovar 1 and the rest was *B. melitensis* Rev. 1.
Conclusions: *Brucella* species were identified from all isolates of sheep and goats of Mongolia. The strains were determined as *B. melitensis* by AMOS-PCR, but other PCR methods showed different results. Two out of the isolates were B. *abortus*, not *B. melitensis*. Multiplex and real-time PCR are more specific for differentiation of species. Besides, 9 isolates were *B. melitensis* vaccine strain Rev. 1. This vaccine strain can cause abortion in pregnant animals.

010P
Colonization kinetics of a lipoprotein 28 deficient mutant of *B. abortus* S19
A.M. Dougherty[1], G. Vernati[1], J. Leonhardt[1], J.E. Lowry[2], G. Andrews[1]; [1]Veterinary Sciences, University of Wyoming, Laramie, WY, USA, [2]Department of Clinical Investigations, Eisenhowser Army Medical Center, Fort Gordon, GA, USA.

Gram negative bacteria have been shown to undergo outer membrane vesiculation (blebbing), which has been generally recognized as a ubiquitous process that occurs in vitro and in vivo. In pathogenic species, it has been also suggested that this phenomenon is linked to virulence. Enterotoxigenic *E. coli* (ETEC) outer membrane vesicles (OMVs) have been shown to carry virulence effectors as part of their cargo. In this pathogen, lipoprotein 28 (NlpA), located in the inner membrane and periplasm, is thought to play a role in the biogenesis of OMVs in this pathogen. A transposon mutant of *nlpA* in ETEC produces less outer membrane vesicles than wild-type strains and thus may be compromised for virulence. To assess the role this cell envelope lipoprotein may play in *B. abortus* in vivo survival and virulence, we identified the *nlpA* homolog in *B. abortus* S19 and subsequently generated a marked insertion mutation by allelic exchange to inactivate the gene. Kinetics of in vitro growth in broth of the *nlpA* mutant and S19 showed comparable growth rates, indicating that viability of the mutant was not compromised. Thirty BALB/c mice were next infected i.p. with 1.25×10^4 CFU of S19*nlpA* and splenic bacterial loads quantitated at 7, 14, 21, 28, 42 and 70 days post-infection, and compared to its isogenic parent. Although not statistically significant, colonization with the *nlpA* mutant appeared to be more rapid than S19. More remarkably, although both the S19 parent and mutant were cleared at the same rate, a significantly higher level of organisms were maintained in splenic tissues at day 70-post infection ($p=0.037$). We posit that the in vivo colonization properties observed with S19*nlpA* may be related to an alteration in the mutant's vesiculation phenotype. We are currently testing this hypothesis in vitro.

011P
Motility of Filamentous Cells of Salmonella enterica
J. Tsarouha[1], N. Faith[1], C. Kaspar[2], A. Wong[2], C.J. Czuprynski[1];
[1]University of Wisconsin - Madison, Madison, WI, USA, [2]University of Wisconsin, Madison, WI, USA.

Salmonella forms filaments when incubated in a high salt concentration. These filaments can invade Caco-2 intestinal epithelial cells in vitro, and colonize and cause systemic infection following intragastric inoculation into mice. In this study we investigated septation of Salmonella filaments into single cells and the motility of filamentous and non-filamentous. Salmonella divided into individual cells within a relatively short incubation period in DMEM with 10% FBS. During a 4-hour incubation, an initial inoculum of 40 µg/ ml wet weight non-filamentous cells increased a little more than one log10 CFU (1.67 x 10^7 to 2.7 x 10^8 CFU), whereas in the same time frame the CFU of filamentous cells increased from 2.7 x 10^5 to 1.4 x 10^8 CFU. Microscopic examination revealed that the greater increase in CFU for the filamentous cells reflects, in part, fragmentation of the filaments into numerous individual cells. By 4 hours, most filamentous cells had divided into smaller rods that migrated further on swimming agar than control cells. These findings indicate that Salmonella can form filamentous cells in response to stress. These filaments in turn can divide into many individual cells that are motile and can facilitate spread of Salmonella in a contaminated food product.

012P
Identification of immunogenic *Brucella canis* outer membrane proteins.
A. Heredia-Antúnez[1], G. Palomares-Resendiz[2], E. Díaz-Aparicio[2], F. Suárez-Güemes[1], **B. Arellano-Reynoso**[1];
[1]Faculty of Veterinary Medicine,, National University of Mexico, Mexico, D.F, Mexico, [2]CENID-Microbiología, INIFAP, Mexico, D.F, Mexico.

Brucella canis, the causative agent of canine Brucellosis, is a rough bacterium which causes abortion, orchitis and epididymitis, as well as infertility. This disease would limit the reproductive capacity in dog kennels. The agglutination test is useful for routinary diagnostic, because it is a rapid and non expensive method; nevertheless, nonspecific crossreaction may occur. Thus, it is important to evaluate different specific antigens as tools for Brucellosis diagnostic. The objective of this work was to identify immunodominant *B. canis* Outer Membrane Proteins (OMPs), as useful specific candidates for serological diagnosis, during canine Brucellosis in dogs naturally or experimentally infected. OMPs were extracted from a wild type *B. canis*, and electrophoresis in a SDS gel was performed, then transferred to a PVDF membrane. Western blot was carried out with sera from nine experimentally infected dogs, and 16 naturally infected dogs. 18 kDa, 28 kDa, 31 kDa, 55 kDa, 72 kDa and 85 kDa OMPs showed constant reaction with sera from both naturally and experimentally infected dogs, in this case at 1 month after infection. We propose those proteins as antigen candidates for further studies concerning specific serological tests for canine Brucellosis.

BACTERIAL PATHOGENESIS POSTERS

013P

Survival and virulence of Salmonella spp. in poultry feed

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Feed has been recognized as a source of *Salmonella* to chickens. However, feed components have very low water activity, and thus the mechanisms of *Salmonella* survival and subsequent colonization of poultry is unknown. Therefore, the purpose of this research was to compare the ability of *Salmonella* serovars and strains to survive storage in broiler feed and to determine the molecular mechanisms associated with survival and colonization by measuring the expression of genes associated with colonization (*hilA, invA*) and survival via fatty acids synthesis (*cfa, fabA, fabB, fabD*) over 7 days in storage. Whole cracked corn was inoculated with one of 15 strains of *S. enterica* consisting of 11 serovars (*S. Typhimurium, S. Enteriditis, S. Kentucky, S. Seftenburg, S. Heidelburg, S. Mbandanka, S. Newport, S. Bairely, S. Javiana, S. Montevideo* and *S. Infantis*). To inoculate corn, cultures were suspended in phosphate buffered saline (PBS) and survival was evaluated by plating samples onto XLT4 agar plates at time points (0h, 4h, 8h, 24h, 4d and 7d). To evaluate gene expression, RNA was extracted from the samples at the time points (0, 4, 8 and 24h) and gene expression measured with real time PCR (qRT-PCR). The survival ability in feed was dependent on the strain, with *S. Enteritidis*(WT) and *S. Typhimurium* ATCC 23595 (LT2) demonstrating the longest survival rates (7 days). In relation to gene expression, *S. Seftenburg, S. enterica* 13076 and *S. Montevideo* exhibited the highest upregulation in the target genes (*hilA, invA, fabA, fabB, fabD*) relative to the other strains. Correlation analysis was performed between survival and gene expression. A low positive coefficient of correlation was obtained between bacterial survival and the genes *cfa, fabA* and *fabB* and for the genes *invA, fabD* and *hilA* a low negative correlation was found in comparison to survival capability of the *Salmonella* strains tested. From this experiment, the data indicates the ability of strains to survive over time in poultry feed was serovar and strain dependent Furthermore, the data indicate that the upregulation of short chain fatty acid synthesis and down regulation of colonization genes may be associated with survival in the poultry feed.

014P

Analysis of the cecal microbial profiles of commercial layer chickens with *Escherichia coli*-induced peritonitis.

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Peritonitis caused by avian pathogenic *Escherichia coli* (APEC) is considered as a major disease problem affecting laying hens due to its negative economic impact on the commercial table egg industry. Despite its economic significance, pathogenesis of *E. coli* peritonitis has not been elucidated yet. Since *E. coli* is a normal inhabitant of the chicken gut, ascending fecal contamination from the cloaca and bacterial translocation from the intestinal lumen are considered possible routes of infection. Recent research has demonstrated the important role that the normal gastrointestinal microflora plays in animal health and nutrition, and that the gut microbial imbalance (dysbiosis) can favor the overgrowth of potentially pathogenic bacteria.
The current study was carried out to ascertain if there is any relationship between peritonitis and cecal microbial profiles of the affected chickens using a culture independent method. Specifically, PCR-denaturing gradient gel electrophoresis (DGGE) with universal primers targeting V6-V8 region of the 16S rRNA gene (approximately 400 bp) was employed to compare the overall cecal bacterial composition of commercial egg layers having peritonitis (n=15) with their healthy counterparts (n=15). The DGGE profiles demonstrated significant differences between sick and healthy groups ($P < 0.05$) yielding less number of bands for cecal samples collected from sick birds compared to that of the healthy birds. We conclude that the chickens with peritonitis have cecal dysbiosis which might predispose them to APEC-induced peritonitis. As such, management of microbial ecology of the intestinal tract may be an important element of preventing peritonitis caused by APEC.

015P

Bacterial community profiling of tonsils from diseased pigs using terminal restriction fragment length polymorphism analysis

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The past decade has seen a number of culture-independent techniques normally used in microbial ecology being applied to profile the bacterial communities of human and animal body sites. Terminal restriction fragment length polymorphism (T-RFLP) analysis, a relatively fast and inexpensive example of these techniques, was evaluated for its ability to detect primary and opportunistic pathogens present in the tonsil of the soft palate in swine and the results compared with conventional culture methods. As part of a larger study on risk-based surveillance of respiratory infections in growing pigs, tonsillar samples were obtained from unthrifty animals in closeout groups (n=127 animals on 28 farms) in finisher facilities. Routine microbiological analysis was performed and both culture (n= 307) and tissue (n=127) samples were characterized by T-RFLP analysis using a Phusion® Bacterial Profiling kit. Samples from 28 healthy swine were also collected and analyzed for comparison. Every step of the analysis, from DNA extraction to peak identification, was optimized. Statistical analyses including cluster analysis, principal component analysis, and correlation analysis were performed to evaluate the relationships of the communities within and between farms and with the clinical and culture data. Using a custom in silico T-RFLP matching database based on species reported in previous studies of the swine tonsil, 68 putative identifications were made to the genus level. The microbial communities of the 128 animals analyzed clustered into 4 groups. In the first group, 65 different genera were putatively identified with Clostridium sp. being most prevalent. The second group contained 57 putative genera with Streptococcus sp. being the most prevalent. Groups three and four were primarily made up of bands that did not have obvious identifications. In conclusion, T-RFLP analysis is a rapid and relatively cost effect method that holds promise for obtaining a more complete picture of microbial communities than is currently available by routine bacterial culture methods, but definitive identification is not possible.

016P

Expression of adhesin genes of *Actinobacillus suis* grown under conditions that mimic the host environment

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Actinobacillus suis is a common commensal of the tonsil of the soft palate of swine, but under yet to be identified conditions, it can invade the bloodstream and cause septicemia, arthritis, and meningitis. Little is known about its pathogenesis including the critical first steps of host colonization. Thus, the objective of this study was to measure the expression of genes involved in the early stages of attachment to tonsils.
In healthy animals, *A. suis* is thought to exist in the tonsil in biofilm and planktonic forms. Cells in the biofilm form likely persist in a low oxygen and nutrient environment, primarily in the stationary phase of growth. Over time, exponentially growing cells in the planktonic form are shed from the biofilm into a higher nutrient and oxygen environment. We hypothesize that *A. suis* will express different adhesins in these two environments, and that certain signals will cause planktonic cells to assume an invasive phenotype with a different complement of adhesins.
A bioinformatic analysis of the genome of a pathogenic strain of *A. suis* revealed 40 genes encoding 22 putative fimbrial and afimbrial adhesins of interest. Most of these adhesins were similar to ones reported in other *Pasteurellaceae* and include homologues of type IV fimbriae; a *tad* locus; genes encoding tangled pili, prepilins, and a fibronectin-binding protein; 11 outer membrane proteins; and 5 autotransporters.

016 (continued)
To mimic growth conditions in healthy and stressed (i.e., more susceptible to disease) animals, *A. suis* was grown in rich (BHI) and minimal (RPMI+FBS) media, with and without epinephrine, and growth kinetics were assessed. In BHI, doubling times in the presence and absence of epinephrine were 26.7 (R^2=0.9973) and 24.1 (R^2=0.9891) min, respectively, while in RPMI+FBS they were 33.0 (R^2=0.9997) and 31.9 (R^2=0.9916) min, suggesting that under the conditions examined, epinephrine does not appear to significantly affect the growth rate of *A. suis*.
Having established that differences in adhesin expression are not likely to be merely a reflection of differences in growth rate, 10 putative adhesin genes are being assessed for expression in vitro under various conditions that simulate the environment in the host.

017P
Molecular characterization of a surface protein endowed with endonuclease activity related to the restriction enzymes of the RE_*AlwI* superfamily in *Mycoplasma meleagridis*
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Purpose: *Mycoplasma meleagridis* accounts amongst the four major avian pathogens, along with *Mycoplasma gallisepticum*, *Mycoplasma synoviae*, and *Mycoplasma iowae*. However, despite its pathogenicity, *M. meleagridis* antiginicity remains not well explored and its surface proteins have not been extensively studied. To address this deficiency, we report on the identification and the characterization of a *M. meleagridis* surface protein associated with an endonuclease activity.
Methods: We screened a λ phage expression library of *M. meleagridis* genomic fragments with an anti-*M. melagridis* serum that had been pre-adsorbed with whole cell extracts of 6 other avian *Mycoplamsa* species and had been depleted of antibodies to the cytosolic fraction. The identified *M. meleagridis* DNA insert, Mm19, of the λgt11 clone 19 was sequenced, edited and analyzed. Surface-bound endonuclease activity was demonstrated by incubating a closed circular plasmid DNA with a *M. meleagridis* cell suspension. An antiserum produced against the bacterially expressed gluthatione sulfotransferase fusion of Mm19 was used to confirm the surface location of Mm19 and its endonuclease activity.
Results: We identified a partial ORF, referred to as Mm19, which revealed a significant sequence similarity with the RE_AlwI superfamily. Mm19 showed significant homologies with AlwI related sequences in other mycoplasmas and other bacterial species. The fact that plasmid circular DNA was fully degraded when mixed with *M. meleagridis* whole cells and did not when these cells were pre-incubated with antibodies to Mm19, indicated that *M. meleagrids* surface-bound endonuclease activity is associated with Mm19.
Conclusions: We report, for the first time, on the identification of a surface-bound endonuclease activity in *M. meleagridis* related to the Alw1 superfamily of restriction endocucleases.

018P
A fatal case of Arcanobacterium pyogenes in 9-year-old Korean native cattle
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A 9-year-old, female Korean native cattle was submitted to Animal Plant and Fisheries Quarantine and Inspection Agency for diagnostic investigations after sudden death without any premonitory symptoms.
At necropsy, solid cystic nodule containing white-to-yellowish purulent exudate was observed from cerebrum, heart, liver, spleen and kidney. Histopathologically, fibrinosuppurative encephalitis, hepatitis, myocarditis and pyogranulomatous interstitial nephritis were observed from brain, liver, heart and kidney accompanied by multiple sites of mineralization with infiltration of neutrophils and fibrin.
As the tissue appeared to be positive for gram stain and the Arcanobacterium pyogenes specific gene detected by PCR, we diagnosed this case as arcanobacterium infection. We excluded the possibility of bovine tuberculosis infection which resembles the histopathologic lesion of arcanobacterium infection by the negative result of acid-fast stain and PCR.
The present study appears to be the first case report of Arcanobacterium pyogenes with multiple abscesses in Korean native cattle.

019P
Prevalence of Coxiella burnetii in a healthy bighorn sheep population
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Coxiella burnetii is a Gram-negative bacterium that causes the zoonotic disease Q fever. Because it is highly infectious and durable in the environment, C. burnetii has been classified as a category B bioterrorism weapon and a select agent. Ruminants are a major reservoir and though infections are usually asymptomatic, increased abortion rates have been observed, particularly in goats. C. burnetii forms a stable small cell variant that can survive for long periods of time in the environment, where prevalence rates have been estimated as high as 44.6% in the Rocky Mountain area. Because of the far-reaching ramifications of C. burnetii infection, its ability to be easily aerosolized, and its durability in the environment, prevalence in wildlife ruminant populations may be an important part of the disease cycle. Bighorn sheep have been identified as a possible wild reservoir of C. burnetii, with infection rates estimated at 10% in southern California. This study used a PCR assay to test a healthy population of bighorn sheep for the presence of C. burnetii. Fecal samples were collected from 60 healthy bighorn sheep during relocation of three groups in Nevada. Samples were submitted to the Iowa State University Veterinary Diagnostic Laboratory for PCR testing. The presence of C. burnetii was tested by PCR of the IS1111 repetitive element. Of the 60 animals tested, one was positive for C. burnetii, representing an infection rate of 1.6%. In this small study, C. burnetii is currently not an important pathogen among bighorn sheep. However, co-mingling of domestic livestock with wildlife can result in negative health implications. Efforts to reduce or prevent contact between wildlife and domestic livestock populations may decrease the risk of transmission of devastating pathogens. Further surveillance and testing of these populations is merited based on these data.

020P
Zebrafish larvae as model to evaluate lipopolysaccharide toxicity
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Bacterial lipopolysaccharides (LPS) or endotoxins are structural components of the outer membranes of Gram-negative bacteria and also potent inducers of inflammation in mammals. Unregulated inflammatory response to LPS can lead to septic shock syndrome, a pathological condition with manifestations such as hypotension, acute respiratory distress syndrome, disseminated intravascular coagulation and multiple organ failure. Higher vertebrates are extremely sensitive to endotoxin even at low doses but lower vertebrates like fish are resistant to the toxic effects of LPS. However, it has been determined that zebrafish (Danio rerio) larvae respond negatively to bacterial LPS exposure. LPS consists of lipid A, a polysaccharide with an inner and outer core and a highly variable O-antigen portion composed of oligosaccharide subunits. Lipid A, the minimal structure of LPS with stimulatory activity, consists of a

020 (continued)
C12-14 fatty acids linked to a phosphorylated N-acetylglucosamine dimer. The composition and number of these N-acyl side chains are important in the activation of the immune inflammatory response, as different lipid A structure vary considerably in potency. In this work, we explored the use of zebrafish larvae as a model to study LPS toxicity. Three-day post fertilization larvae were expose to different concentration of LPS from different bacterial species. We determined that LPS from bacterial fish pathogens like Edwardsiella ictaluri and Flavobacterium columnare has low killing effect. In contrast, Salmonella Typhimurium LPS possesses a high killing effect. We also evaluated different S. Typhimurium LPS that have a detoxifying lipid A structure. We concluded that zebrafish might be a good model for studying endotoxin toxicity.

021P
Application of Raman spectroscopy in antimicrobial drug discovery research
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Antimicrobial drug development is a time-consuming and costly process. Much of the difficulty originates in identifying the mechanism of action (MOA) of new potential antimicrobial compounds. Therefore, the ability to quickly identify MOA of new compounds is a critical development for antimicrobial drug research. Raman spectroscopy (RS) has been identified as a powerful tool for analyzing bacterial phenotype due to its sensitivity, short analysis time, and non-destructive nature. In this study RS was used to get insight into the MOA of a thiazole antimicrobial compound with an unknown MOA. Escherichia coli were subjected to ten antimicrobials, with known MOAs, and the unknown compound at three times the minimum inhibitory concentration for 30 min before being analyzed by RS. Raman spectra were collected using 532 nm laser with 10 mW power and 25 s exposure time. An average of 84 spectra were collected for each treatment. The collected spectral data were used to create a Discriminant Analysis (DA) model. DA was able to discriminate samples based on drug treatment with 93.2% accuracy. The DA model was then used to identify antimicrobials that have a similar MOA to the unknown compound. The model shows that the phenotype produced under the effect of the unknown compound was primarily similar to that under the effect of RNA polymerase inhibitor, and to a lesser degree under protein synthesis inhibitors. More studies are being conducted to confirm the MOA of the unknown compound. However, results demonstrated the potential for RS as a powerful tool in drug discovery research.

022P
New drugs for bad bugs: class II HMG-CoA reductase inhibitors
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Purpose: This study was designed to screen and initiate testing of new antimicrobial compounds against a novel target, class II HMG-CoA Reductase (HMGR), against resistant organisms methicillin resistant *Staphylococcus aureus* (MRSA) and vancomycin resistant *Enterococcus faecalis* (VRE). The enzyme is part of the mevalonate pathway which is essential to survival for gram positive cocci. Antimicrobial resistance is a growing concern in medicine, and existing antibiotics are limited in the molecules they target. New antibiotics that target novel pathways and enzymes within resistant pathogens needed to be developed to combat such bacteria.
Methods: A high throughput screen selected a lead compound with low micromolar inhibitory activity towards the bacterial HMGRs. This molecule became the basis for a series of analogues designed by our group that. In this study, 22 novel bacterial HMGR inhibitors were tested for cytotoxicity and inhibition of *S. aureus* HMGR. The compounds were screened against MRSA and VRE. Effective compounds were further tested to determine minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC). A unique whole animal infection model using the nematode *Caenorhabditis elegans* as a host for MRSA and VRE was screened for anti-infective properties of HMGR inhibitors in an environment that better approximates an infected human host than current *in vitro* models.
Results: The compounds showed they were not cytotoxic at 100 µMol and effectively inhibit *S. aureus* HMGR. Of the 22 compounds, 13 were active against MRSA and 9 were active against VRE at micromolar concentrations. Six of the tested compounds appear to be bactericidal *in vitro* against MRSA, and three are bactericidal to VRE.These compounds were also more effective than the current drug of choice, vancomycin for MRSA and linezolid for VRE, at reducing bacterial load in *C. elegans*, and are not toxic to healthy worms.
ñConclusions: Class II HMG-CoA reductase inhibitors show promise in treating these resistant pathogens and warrant further investigation and testing.

023P
Use of matrix-assisted laser desorption/ionization time-of-flight mass spectrometry to detect *Streptococcus suis* variants
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Streptococcus suis is a common pathogen in swine farms worldwide and is associated with a variety of diseases such as meningitis, arthritis, bronchopneumonia, and septicemia in piglets. A tentative diagnosis often can be made on the basis of history, signs, lesions and the demonstration of Gram-positive cocci in the lesions. However, *S. suis* can be isolated from normal, healthy pigs and other *Streptococcus* species are common in swine. Confirmation should be made through culture and identification of the streptococci. Traditionally, *S. suis* identification is based on colony morphology, biochemical reactions and serotyping. However, results from these traditional methods are variable and therefore diagnosis based purely on these reactions is sometimes difficult. Molecular tests such as polymerase chain reaction or 16S ribosomal RNA (16S rRNA) sequencing are available in some labs, but the cost is generally higher than conventional biochemical tests. In the last few years matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) has been increasingly studied and applied for the identification and typing of microorganisms. MALDI-TOF MS has been introduced in clinical routine microbiological diagnostics with marked success in terms of accuracy, speed, cost-effectiveness and ease of use. In this study we compared the identification of *S. suis* isolates by conventional biochemical reactions, serotying, MALDI TOF MS and 16S rRNA sequencing. We found that MALDI TOF MS was able to consistently identify *S. suis* isolates that reacted in an expected manner with conventional testing, but MALDI TOF MS was also able to identify as *S. suis* isolates with unusual colony morphology, variable biochemical reactions or unclear serotyping results. Additionally, MALDI TOF MS results were more likely to agree with results from 16s rRNA sequencing than from conventional methods. These results indicate that MALDI TOF MS may be a better method to identify *S. suis* variants that may have previously been considered as insignificant or contaminant growth.

BACTERIAL PATHOGENESIS POSTERS

024P

Detection and identification of a new species of *Mycoplasma* in swine

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Following abortion and higher mortality rates at the farm, an adult dying sow was euthanized and submitted for necropsy at the pathology laboratory of the Faculté de médecine vétérinaire of Université de Montréal. Haemorrhage was noted in trachea, heart and lung and the foetuses found in the uterus had marked post-mortem changes. The vessels of many organs of the sow including lung, liver and spleen contained numerous monocytes and some of them showed erythrophagocytosis. *Streptococcus suis* was found in spleen and lung tissues.
Foetus tissues were tested by PCR and negative results were obtained for swine Parvovirus, *Leptospira spp*. and porcine reproductive and respiratory syndrome virus (PRRSV). A positive result was obtained for *Mycoplasma spp*. A PCR assay targeting the 16S ribosomal RNA gene was realized and the obtained PCR product was sequenced for further characterization. Analyses by BLAST gave the highest nucleotide homologies to "*Candidatus Mycoplasma turicensis*" (92%) and to *Mycoplasma haemofelis* (91%), which are two hemotropic mycoplasmal species described in cats. Therefore, a Real-Time PCR assay was developed from the 893 nucleotide sequence obtained for further investigation. Whole blood samples collected from animals housed at the same farm of the initial clinical case gave positive results with low Ct values, suggesting a high amount of genomic DNA. However, blood smears followed by acridine orange staining, for the visualization of bacteria such as mycoplasma, were inconclusive. Of note, blood samples collected from other farms gave negative RT-PCR results.
Until now, all attempts to isolate this new bacterial species were unsuccessful. Electron microscopy was done on thin sections of fixed blood cells and revealed the presence of intracellular particles within the macrophages with mycoplasmal-like shapes. Further investigation is needed to access the prevalence of this new bacterial species and its involvement in swine disease.

025P

Evaluation of diagnostic tests for the assessment of human brucellosis in Georgia

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Purpose: *Brucella* species cause substantial morbidity in the human population and agricultural economic loss in Georgia; and frequently as in other countries, cases are underreported. The present study aims to improve the brucellosis surveillance system in the Country of Georgia with an emphasis on determining the diagnostics most suitable for quick and accurate
laboratory-based identification of human cases. Methods: Volunteers ($\geq$18 y/o) with symptoms suggestive of brucellosis were enrolled in this prospective investigation. Clinical as well as epidemiological information and blood specimens were collected at initial and follow-up visits for 80 individuals. Samples were subjected to serological testing and blood culture.Results: Analysis of collected information has shown that in 50% (40/80) of the suspected brucellosis cases clinical diagnosis was supported (titer $\geq$ 200) by routine serological laboratory tests, such as slide and tube agglutinations (SAT/TAT). Among these 40 seropositive probable cases diagnosis was confirmed by *Brucella spp*. culture isolation from 27 individuals. In addition, six of the patients initially negative by serological testing were later *Brucella*-confirmed by culture isolation and seroconversion. Overall, 57.5% (46/80) of enrolled suspected cases had laboratory-diagnosed brucellosis, whereas only 41.2% (33/80) were confirmed by recovering a *Brucella* isolate from blood culture. Negative sera was subjected to further investigation; samples negative by agglutination and culture were tested for the presence of anti-*Brucella* total immunoglobulin (Ig), and immunoglobulins M (IgM) and G (IgG). Total antibody together with IgG was obtained in 62% (21/34), whereas IgG only and IgM were detected in 9% (3/34) and 6% (2/34), respectively.
Conclusions: The above data suggests that the tube agglutination test, the major clinical laboratory diagnostic method used in Georgia, is not capable of detecting the majority of chronic cases of brucellosis and should be supported by other tests for accurate diagnosis.

026P

Investigation on the diagnostic efficiency of STAT for brucellosis

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Purpose: To investigate the diagnostic efficiency of Standard Tube Agglutination Test (STAT) used as confirmatory serological test for brucellosis in South Korea, there were compared and evaluated with three serological confirmatory tests such as the Indirect-ELISA (I-ELISA), Competitive-ELISA (C-ELISA) and Fluorescent Polarization Assay (FPA) in this study.
Methods: A total of 345 bovine serum samples diagnosed as brucellosis-positive or -suspected serum by the RBT (Rose-Bengal test) and the STAT were collected from regional veterinary branch under national brucellosis monitoring program from 2010 to June 2012 in South Korea. For comparison with STAT, three serological tests were performed and evaluated according to manufacturer's instruction.
Results: In the STAT, 302 of 345 (87.5 %) bovine serum samples were diagnosed as positive, and in I-ELISA, C-ELISA and FPA, 215 (62.3 %), 223 (64.6 %) and 194 (56.2 %) serum samples were also proved to positive, respectively. The STAT showed quite high positive result as compared with three confirmatory prescribed tests from OIE.
Conclusions: Because the STAT is estimated to have many false-positive results as a confirmatory method, so more accurate serological tests such as ELISA and FPA are required to confirm brucellosis in South Korea.

BIOSAFETY AND BIOSECURITY POSTERS

027P

Experience of implementing international recommendations for control recombinant DNA safety in Ukraine

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Purpose: Despite the large number of controlling DNA vaccines orders, most of them serve as guidelines. Consider actual requirements documents, DNA preparations recommendations of the OIE and the WHO, our experience of research the recombinant DNA technologies, the state standards of Ukraine "Molecular diagnostics. DNA-vaccine. Methods of quality control" (analog state SOP) was developed by us. Methods: PCR, RFLP, electrophoresis, sequencing, spectrophotometry, microbiology, LOL-test, MTT-test. Results: The proposed control system of quality DNA vaccines for veterinary use involves the following steps: a) Authenticity control of the specific gene region in plasmid DNA (PCR). b) Availability control of the specific gene insertion in the plasmid material (RFLP). c) Uniformity control of the plasmid DNA (electrophoresis). d) Nucleotide sequence control of the specific gene insertion in the plasmid DNA (sequencing). e) Concentration and purity control of the plasmid DNA (spectrophotometry). f) Microbial contamination control of the DNA vaccine. g) Safety control of the DNA vaccines (testing in laboratory animals). h) Availability control of the bacterial endotoxins in DNA vaccines (LOL-test). i) Cytotoxicity control of the DNA vaccines (MTT-test). j) Immunogenicity control of the DNA vaccines (ELISA). k) Protective effectiveness control of the DNA vaccine (testing in laboratory animals). Conclusions: Developed standard is a first Ukrainian experience of implemintation international biosafety recommendations for recombinant DNA technique in the practice of the biotechnological production.

028P

Study of the effect of metallic nanoparticles on the growth properties of discrete and associated forms of *Pasteurella multocida*

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Purpose: The purpose of research. To study the antimicrobial properties of nanoparticles of metals silver, copper, zinc, manganese dioxide and iron in the range of concentrations on the museum test strain *Pasteurella multocida* and it' association.
Methods: We used nanoparticles of metallic silver (Ag), copper (Cu), zinc (Zn), manganese dioxide (Mn) and iron (Fe), and the *Pasteurella multocida museum test strains "7"*, Salmonella choleraesuis *museum test strains "12"*, Streptococcus suis *museum test strains "34"*. Microorganisms were grown on classical bacteriological media containing colloidal dispersions of nanoparticles of Ag, Cu, Zn, Mn and Fe, taken at the final concentrations (for metal) 200, 80, 40, 4, 0.4, 0.04, 0.004, 0, 0004 microgram/ml. Control is bacteria cultures which grown without nanoparticles.
Results: There was found that nanoparticles of Ag and Mn in the studied concentration range showed bacteriostatic effect on the *P. multocida* growth. In the presence of Cu, Zn, Fe nanoparticles (from 0.04 mcg/ml) there was stimulation of growth discrete forms of *P. multocida* . But associative forms of *P. multocida* reproduction was inhibited by 0.0004mcg/ml of these nanoparticles with simultaneosly 2%activation of growth of S.choleraesuis. Exposure of *P. multocida* association with St. suis, S.choleraesuis in Fe presence (from 0.04 to 200 mcg/ml) inhibited the total accumulation of bacterial mass. But Fe in 0,004-0,0004 mcg/ml concentrations induced 2% increase in biomass of all strains of bacterial association. Zn nanoparticles (0,04-0,0004 mcg/ml) have activated reproduction of all parts of association. Mn nanoparticles (0.0004 mcg/ml) selectively stimulated the accumulation of *P. multocida* and *S.choleraesuis* biomass, whereas it oppressed *St.suis* throughout the whole range of concentrations. Increase of the Pasteurella growth is closely correlated with the growth of their antibiotic resistance.
Conclusions: The results indicate the importance of environment metallic pollutions for the Pasteurella maintenances and it's antibiotic resistance formation.

COMPANION ANIMAL EPIDEMIOLOGY POSTERS

029P

Veterinary Epidemiology of Rabies in Ukraine

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Purpose: In Ukraine in spite of considerable financial expenses on oral immunization of foxes and parenteral immunization of dogs and cats, it is not succeeded to reach considerable results in the fight with rabies. Unfortunately there was an negative tendency to increasing a part of dogs and cats in the structure of rabies disease which are the main source of rabies in people. That's why the purpose of the research was to find out antropurgisation of rabies in Ukraine.
Methods: Analysis of 228 anamnesis data of dogs infected with rabies and sent to the Institute of Veterinary Medicine during 2008-2012.
Results: Analysis of animal morbidity on rabies in Ukraine in period of 2006-2011 found out the changes of specific structure of morbidity that means decreasing a part of wild animals (from 49,0 % in 2006 to 38,7 % in 2011) and increasing a part of dogs (from 18,3 % in 2006 to 23,2 % in 2011) and cats (from 19,8 % in 2006 to 25,0 % in 2011) in the general amount of animals which perished from rabies.
A lot of Ukrainian scientists and doctors of veterinary medicine consider that the main reason of spreading the rabies is a great number of homeless animals which factually are the reservoirs of infection in towns and villages.
However, in our opinion spreading of rabies shows the insufficient level of measures of control of rabies among home animals. It was confirmed with conducted analysis and set that only 26 (12,9 %) dogs were vagrant, others 202 (87,1 %) had owners, but didn't get necessary protective rabies vaccination. According to Ukrainian instruction "Preventive measures against rabies of animals", all the dogs must be vaccinated in spite of the existence of rabies in this or that region, but it actually appears it is quite not so.
Conclusions: Got results were sent to the State committee of veterinary medicine of Ukraine and will be the argument for strengthening of control after conducting rabies vaccination of dogs and cats. So the conducted analysis expressly demonstrates that at present problems eradication of rabies in Ukraine is impossible as the problem of homeless animals is not solved, which next to foxes, remain the important source of contagium, and the positions of instruction are also not executed.

030P

Escherichia coli with CTX-M-15 type ESBL isolated from urinary samples of dogs and cats

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Purpose: Extended-spectrum β-lactamase- (ESBL) producing *E. coli* have emerged in human and veterinary medicine and are thereby described to cause urinary tract infections. The aim of this study was to investigate the presence of ESBL-producers among uropathogenic *E. coli* isolated from dogs and cats and to further characterize detected ESBL-producing isolates.
Methods: A total of 107 *E. coli* strains isolated from urinary samples of companion animals admitted to a veterinary hospital were investigated. Isolates were tested for their antimicrobial susceptibility using the VITEK 2 Compact system and results were interpreted according to CLSI guidelines. Isolates suspicious for ESBL-production were subjected to confirmatory tests using ESBL Etests (CT/CTL, TZ/TZL, PM/PML). ESBL-producing *E. coli* isolates

COMPANION ANIMAL EPIDEMIOLOGY POSTERS

030P (continued)
were further characterized by identification of ESBL-genes (bla_{TEM}, bla_{SHV}, and bla_{CTX-M}), multi-locus sequence typing (MLST), detection of putative virulence genes, and analysis of *E. coli* phylogroups.
Results: Among the 107 *E. coli* isolates (59 from dogs, 40 from cats), eight isolates from four different animals (dog A and B, cat C and D) were found to be ESBL-producers. In addition to being resistant to β-lactams (except carbapenems), resistances to various other classes of antimicrobials were found in these isolates. Further characterization showed that the eight ESBL-producing *E. coli* strains were of ST533/CTX-M-15/TEM/phylogroup B1 (four strains from dog A), ST410/CTX-M-15/TEM/phylogroup A (one strain from dog B, two strains from cat D), and ST648/CTX-M-15/phylogroup D (one strain from cat C). In terms of putative virulence factors, all ESBL-producers harbored *lpf*A, *sat*, and *tsh*, whereas *iss* was only detected in strains of ST533.
Conclusions: ESBL-producers were detected among uropathogenic *E. coli* from companion animals in Switzerland. The eight ESBL-producing isolates belonged to three sequence types (ST410, ST533, ST648), three *E. coli* phylogroups (A, B1, D), and all produced CTX-M-15. For the first time, *E. coli* of ST533 carrying $bla_{CTX-M-15}$ were thereby detected in a dog.

031P
Detection of Mycoplasma gallisepticum natural reservoirs among waterfowl
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The aim of our study was to identify possible natural reservoirs of *Mycoplasma gallisepticum* among decorative and wild waterfowl.
The study was conducted in private poultry farms and wild populations of waterfowl. From bird selected samples of blood serum and egg yolks for research in serum plate agglutination test (SPA) and nasal washings for bacteriological research.
In private zoo "SHF" examined 157 heads of decorative birds, including representatives of the order *Galliformes* (chicken, guinea fowl, pheasants and peacocks), waterfowl (ducks, swans) and parrots. It was found 21.66 % seropositive poultry, including chicken and parrots - 26.47 % waterfowl - 12.73 %. In *Galliformes and* parrots cultures of *M. gallisepticum* were isolated from 18.63% of individuals, in waterfowl - 5,45%.
Similar studies were conducted on a private farm "M". There were examined 178 heads. The highest level of seropositivity noted in chickens and turkeys (22 and 53%), pheasants (about 12%). Number of seropositive birds in the whole was about 19%, including waterfowl - 8.11%. On average, about 18% of individuals were carriers of *M. gallisepticum.* For chicken, this number was 13.48%, for waterfowl - 2.7%.
It was also conducted serological monitoring in populations of wild waterfowl (*Ichthyaetus relictus, Sterna nilotica, Sterna herundo, Casarca ferruginea*) in National Park “Askania Nova” (Crimea). In populations of *Casarca ferruginea* for 3 years revealed a stable trend for the presence of antibodies in the serum of adult birds (average 17%) and the egg yolks (average 12%), indicating that the long circulation of field isolates of *M. gallisepticum* in populations of wild waterfowl.
It is proved that *M. gallisepticum* can persist among decorative waterfowl for her welfare with *Galliformes*, while waterfowl is a reservoir of the pathogen. Also natural reservoirs of *Mycoplasma* can be wild waterfowl (*Casarca ferruginea*). Such groups (populations) of birds may serve as a source of infection for commercial herds.

EPIDEMIOLOGY AND ANIMAL HEALTH ECONOMICS POSTERS

032P
Efficacy of a lacteal-derived colostrum replacer feeding program for preventing failure of passive transfer in calves.
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Purpose: Pooled colostrum-related failure of passive transfer (FPT) in calves can be prevented by feeding alternative sources of IgG in the form of colostrum replacement products. We evaluated the efficacy of a commercial lacteal-derived colostrum replacer for prevention of FPT in calves in a large dairy where calves were normally fed pooled maternal colostrum.
Methods: Calves were randomly assigned to be fed either 3.8 L of pooled maternal colostrum or 2 doses (200 g of IgG) of a lacteal-derived colostrum replacer. Blood samples were collected from each calf prior to feeding the colostrum and approximately 24 h after colostrum intake. Serum IgG and total protein concentrations were quantified using standard methods. The apparent efficiency (AEA) of IgG absorption was calculated.
Results: Serum TP and IgG concentrations at 24 h were significantly lower for calves fed pooled maternal colostrum (Mean ± SD; TP = 4.77 ±0.55 g/dL; IgG = 7.50±5.0 g/L) compared with calves fed the lacteal-derived colostrum replacer (Mean ± SD; TP = 5.50 ± 0.52 g/dL; IgG = 15.15±4.75 g/L) product. The AEA was significantly higher in calves fed pooled maternal colostrum (Mean ± SD; AEA = 36.55 ± 26.97%) compared to the lacteal-derived colostrum replacer fed group (Mean ± SD; AEA = 29.33 ± 9.55%). Feeding lacteal-derived colostrum replacer (vs. pooled maternal colostrum) was associated with a 0.73 g/dL (b = 0.73; 95% CI: 0.64 to 0.82) and 7.65 g/L (b = 7.65; 95% CI: 6.84 to 8.46) increase in 24 h serum TP and IgG concentrations respectively. The AEA of IgG absorption was 7.23% (b = − 7.23; 95% CI: −11.08 to −3.38) lower in calves fed the lacteal-derived colostrum replacer compared with pooled maternal colostrum. The odds of FPT decreased by 95% in calves fed the lacteal-derived colostrum replacer compared with calves fed pooled maternal colostrum (OR = 0.05; 95% CI: 0.03 to 0.08).
Conclusions: These findings indicate that feeding pooled maternal colostrum significantly increases the risk of failure of passive transfer of immunity in calves. The lacteal-derived colostrum replacer evaluated is a viable alternative for enhancing adequate passive transfer of immunity in calves.

033P
Transmission of *Brucella abortus* in calves younger than 3 months diagnosed using the card and the immunodiffusion tests in two dairy herds in the state of Queretaro.
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Purpose: The transmission of *Brucella abortus*, in one week and three month old calves born from positive or from negative cows, was determined using the card test as a screening test and the radial immunodiffusion (RID) test as a confirmatory one.
Methods:We worked on two herds, herd 1, had 670 milking cows and a seroprevalence to brucellosis of 21.6% (145/670). In this herd, we formed the group of positive cows that had calved female calves (n=22) from which 2 (9.1%) were positive using the RID test at one week and three months of age. In the group of negative cows that had calved female calves (n=22), all calves were negative to brucellosis at one week of age and 4 (18.2%) were positive using the RID test.
Results: . We collected 20 milk samples from the cooling tank of the controlled production unit were the cows positive to *Brucella* were milked obtaining the isolation of *B. abortus* in 100 % of them and making confirmation by means of PCR test that it was a field strain. Herd 2, had 1,800 milking cows and it was participating in the National Campaign against animal Brucellosis of SAGARPA. A seroprevalence of 1.94 % in cows (35/1800) using the card and rivanol tests was detected from January to December 2009. We analyzed 1,170 records from calves younger than 3 months of age from January 2009 to

033P (continued)
June 2010. We found 24 (2.1%) calves positive to *B. abortus* using the card and rivanol tests.
Conclusions: It is concluded that the infection of *B. abortus* can spread to calves by different means. The diagnosis must be carried out in calves in order to avoid confusion with post-vaccinal antibodies and to avoid an abortion on the first pregnancy. This was demonstrated on herd 1 where there was a high incidence of brucellosis and where the isolation of field strains of *B. abortus* occurred.

034P
Real time PCR detection of hemotropic *Mycoplasma* species in symptomatic dairy cattle from the Midwest United States
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Purpose: *Mycoplasma wenyonii*, previously *Eperythrozoon wenyonii*, is a non-culturable hemoplasma that infects cattle. In the United States, *M. wenyonii* has been thought to be of low pathogenicity, and reports of clinical disease are rare. An investigation into this organism was initiated in response to an outbreak of clinical disease in multiple dairy cows exhibiting signs previously reported in cattle infected with *M. wenyonii*, including hindlimb edema and reduced milk production. Methods: Blood smears from symptomatic cattle were consistent with *M. wenyonii* infection, however, PCR detection was recommended for confirmation. Several previously published qPCR assays to detect two bovine hemoplasma species, *Mycoplasma wenyonii* and *Candidatus Mycoplasma haemobos*, were validated in our laboratory for sensitivity and specificity and utilized to detect these hemotropic *Mycoplasma* species. Results: Serial samples from symptomatic and normal cattle demonstrated a high prevalence with cyclicity of hemoplasma species infection in this herd. Normal and symptomatic cattle demonstrated equally high prevalence of *C.M. haemobos*, and, to our knowledge, this is the first report of *C.M. haemobos* detection in the United States. Symptomatic animals had a higher prevalence of *M. wenyonii* closest to the incidence of clinical disease than clinically normal herdmates and tended to be more likely to have dual infections with both *M. wenyonii* and *C.M. haemobos*. Over the course of the study, prevalence between the groups for *M. wenyonii* became equal. Conclusions: This work suggests that *M. wenyonii* can cause persistent infection in US cattle, warranting further investigation into the significance of this disease, including its pathogenesis and ecology. Additionally, we demonstrate the necessity of PCR for the sensitive and accurate detection of non-culturable hemoplasma species for investigational studies and diagnostics.

035P
Minimum inhibitory concentrations and antimicrobial resistance patterns of ovine and caprine field strains of *Corynebacterium pseudotuberculosis*
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Purpose: Caseous lymphadenitis (CL) is an important health concern in small ruminants that is difficult to eradicate once it is present in a herd. *Corynebacterium pseudotuberculosis*, the etiologic agent of CL, is a Gram positive, facultatively anaerobic intracellular bacterium. Due to its cell wall fatty acid structure, this organism is resistant to phagocytic attack *in vitro* and to external environmental conditions, making it difficult to treat and to prevent. Internal abscessation can be life-threatening and require appropriate antibiotic treatment; however, antimicrobial susceptibility testing is rarely performed on this organism due to its fastidious nature and lack of CLSI interpretive criteria. Coryneform bacteria such as *C. pseudotuberculosis* have long been thought to be highly susceptible to penicillin antibiotics, yet lack of response has been reported. The purpose of this study was to assess field strains of *C. pseudotuberculosis* for minimum inhibitory concentrations (MIC's) and antimicrobial resistance patterns to commonly prescribed veterinary antibiotics.
Methods: Forty *C. pseudotuberculosis* isolates recovered from sheep and goats with active abscesses were tested using broth microdilution against ceftiofur, tiamulin, chlortetracycline, gentamicin, florfenicol, oxytetracycline, penicillin, ampicillin, danofloxacin, suphadimethoxine, neomycin, trimtheroprim/sulfamethoxazole, spectinomycin, tylosin, tulathromycin, clindamycin, and enrofloxacin.
Results: Using CLSI interpretive criteria for Corynebacterium species, antimicrobial resistance was detected in nearly 50% of isolates to penicillin, over 30% of isolates to tetracyclines, over 20% to cephalosporins, and over 40% to gentamicin. Resistance to clindamycin and enrofloxiacin was rare.
Conclusions: Surveys for antimicrobial susceptibility patterns in organisms that are difficult to identify and test can provide useful information to clinicians initiating treatment for systemic disease as well as monitoring shifts in antimicrobial resistance patterns over time.

036P
Q-fever: epizootic situation and laboratory diagnostics
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Q-fever is a zoonotic disease caused by the ubiquitous pathogen Coxiella burnetii, and is responsible for acute and chronic clinical manifestations. C. burnetii is a category B bioterrorism agent that is highly infectious to both humans and livestock. Farm animals and pets are the main reservoirs of infection, and transmission to human beings is mainly accomplished through inhalation of contaminated aerosols. C. burnetii is a pleomorphic obligate intracellular bacterium that replicates to high number, albeit slowly (Zamboni, Mortara et al.2001), in the phagolysosomes of eukaryote phagocytic cells (Hackstadt and Williams 1981). C.burnetii is incredibly resistant to physical and chemical insult including elevated temperature and pressure, desiccation, osmotic shock and several chemical disinfectants.
The aim of study was to analyze the distribution of Q-fever in the Odessa region of Ukraine and to review the methods of laboratory diagnosis for C. burnetii. Diagnosis of Q-fever is complex and set on the basis of epizootic and epidemiological data, clinical signs, results of serological tests. Investigations were conducted in the Odessa region. Animals were tested serologically by complement fixation (CF) and enzyme-linked immunosorbent assay (ELISA). A total of 722 sera from domestic animals were examined (sheep - 609, goats - 10, cattle - 72, horses - 3, pigs - 1, dogs - 20, cats - 7) during the time period of 2008-2011. Samples ware also tested by real-time PCR for preservation of C. burnetii.
In 2008 in Odessa Region serum samples of domestic animals were tested. 20,0% of seropositive were revealed, in 2009 - 41,2%, in 2010 - 37,4%, in 2011 - 24,5%. Geograraphic distribution analysis showed that in Odessa region the existence of natural foci Q fever was established in five southern districts. Results for detecting DNA of C. burnetii in 10 samples of blood had negative results.Epidemiologic and serological investigations confirmed the transmission of C. burnetii in Odessa region. Further control efforts and laboratory investigations needed to be conducted.

037P
Genetic characterization and phylogenetic analysis of porcine circovirus type 2 field strains isolated from porcine circovirus disease (PCVD) pigs in Korea
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PMWS is one of the most important disease and causes considerable economic losses in pig producing industry in Korea. The majority of PCV2 isolates can be divided into one of two genotypes, known as PCV2a and PCV2b. This study was conducted genetic analysis of PCV2 based on the ORF2 gene to evaluate genetic characterization of field isolates.
Two hundreds and half kidney samples were collected randomly from slaughter house in Incheon, Korea. Thirteen PCV2 samples were detected by RT-PCR. A full-length ORF2 gene of PCV2 was amplified by PCR with forward primer, PCV2-f1(5'-CCA TGC CCT GAA TTT CCA TA-3') and reverse

037P (continued)
primer, PCV2-r1(5'-ACA GCG CAC TTC TTT CGT TT-3'). The PCV2 positive samples of 702bp were used for DNA sequencing. The 13 PCV2 sequences were analyzed together with 17 representative ORF2 sequences reported in GenBank including the strains of PCV2a (AY256455, AY322004, AF086836, AF264042, AF264041) and PCV2b (AY484409, AY864814, EU302140, EF067852, AY188355, DQ629120, DQ629133, AB072302, AY713470, AF109398), the former Korea isolates in 2009 (FJ905468), PCV2c ORF2 sequence (EU148503) and PCV1 (AY193712).
The results of sequencing analysis of PCV2 indicate that there are no differences with FJ905468 and DQ629120. The genotype of samples used was PCV2b and the subgroup was 1A/B. All 13 ORF2 of PCV2 sequence had a genome length of 702 nt and revealed nucleotide indentities ranged between 99~100% compared with the strain FJ905468 isolated in Korea, indicating no significant differences. However, the nucleotide substitution in the ORF2 gene and the deduced amino-acid sequences of ORF2 compared to the other strains were observed.
This experiment suggests that PCV2 isolates from kidneys of slaughtered pigs in Korea scarcely have occurred genomic mutation.

038P
Immunostimulatory boosting effect of anionic alkali mineral complex solution(Barodon®) on FMDVvaccine in pigs
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Barodon®(Barodon-S.F, Korea) has been introduced for its effectiveness as a nonspecific immunostimulator in pigs. Foot-and-mouth disease virus(FMDV) causes an acute vesicular disease in cloven-hoofed animals as pigs and cattle, especially. FMDV has high susceptibility and spreads over long distances rapidly in pigs. FMD broke out in 2010 and has caused problem up to now in Korea. The purpose of this study is to investigate whether it is able to boost ability to stimulate FMDV specific antibody on FMDV vaccine in pigs.
20 pigs(8weeks) were divided into four groups according to concentration of Barodon®. Groups excepted control group(Group A) were fed with Barodon® as Group B(0.025%), Group C(0.05%) and Group D(0.1%). Total experimental period was 9 weeks. Experimental pigs were inoculated FMDV vaccine(Merial, France) twice at 8weeks and 12weeks. Clinic signs were checked for experimental period. Blood samples were collected at 8, 10, 12, 13, 14, 15 and 16weeks for ELISA. FMD antibody test ELISA kit(Prionics, USA) was used to measure antibody titer.
There weren't observed clinic signs and vaccine side effects for experimental period in pigs. FMDV specific antibody titers of experimental pigs were nearby seropositive level. After first vaccine inoculation, antibody titers of experimental pigs decrease gradually for 8-12weeks. After second vaccine inoculation, antibody titers of experimental pigs increase sharply for first week post second vaccine inoculation and tend to increase gradually for 13-16weeks. Group C and Group D were showed antibody response rapidly and strongly comparing with control group. All groups fed Barodon® maintain high antibody titer levels comparing with control group. Also, seropositivities of groups fed Barodon® were high as Group B(40%), Group C(60%) and Group D(60%) comparing with Control group(20%). In this experiment, Antibody titer decreased when FMDV vaccine was inoculated at 8weeks. This result suggest that FMDV vaccine is inoculated after 8weeks in pig. Pigs fed Barodon® retain high antibody titer level. This result showed that feeding with Barodon® had effect to boost ability to stimulate FMDV specific antibody on FMDV vaccine in pigs.

039P
Pathological investigation of multifocal interstitial nephritis from slaughtered pigs in Korea
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Gross lesions of multifocal interstitial nephritis (MFIN), usually called "white spotted" kidneys, are one of the most common condemnation causes of pig kidneys at slaughter house in Korea. Leptospira spp., and other infectious agents such as the PRRSV, PCV2, CSFV have been proposed as being aetiologically linked with interstitial nephritis in pigs. Pigs that infected this kinds of pathological agent show a lower growing rate. The purpose of this study was to characterize the lesions associated to "white spotted" kidneys in pigs at slaughter, and to investigate the prevalence of several infectious agents (Leptospira spp., PRRSV, PCV2 and CSFV).
250 kidney samples from slaughter house in Korea were selected randomly for the present study. Kidneys were pathologically classified from 0 to 3 following the macroscopic criteria. The grading criteria were as follows : grade 0 (no gross lesions), grade 1 (less than 10 whitish foci between 2-5 mm in diameter), grade 2 (more than 10 whitish foci, or presence of one white stain, or more, measuring less than 1 cm in diameter), grade 3 (renal cortical tissue completely covered by whitish foci or stains). Tissue samples from kidneys were maintained in NBF between 24 and 48 hours, and were subsequently dehydrated and embedded in paraffin wax. And these microscopic kidney samples were compared with the macroscopic lesions. For the detection of the infectious agent, the PCR was used. Grade 1 and 2 in macroscopic lesions accounted for 30.8% and 2.4%, respectively. FN (follicular nephritis), ILF (interstitial lymphoplasmocitic foci smaller than a glomerulus), granuloma, PMN (presence of neutrophils) and fibrosis in microscopic lesions were observed frequently in grade 2 than grade 1 in macroscopic lesions. 13 cases of PCV2(5.2%) were observed in total 250 kidneys. 10 PCV2(12.0%) and 2 PRRSV(2.4%) cases were detected in the kidneys showing macroscopic lesions. But other agents were not detected.
In this study, the prevalence of interstitial nephritis from slaughtered pigs in Korea was considered high. The cases of PCV2 and PRRSV were detected. This study suggests that the interstitial nephritis in Korea is closely related to the systemic wasting diseases.

040P
Longitudinal study of fecal contamination of cattle feed by starlings at dairy farms in Ohio
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The European starling (*Sturnus vulgaris*) is responsible for large economic losses to the cattle industry through the consumption of cattle feed and the contamination of the farm environment with droppings that may result in the spread of zoonotic pathogens like *Escherichia coli* O157:H7 (*E. coli* O157:H7). The objective of this study was to understand the temporal patterns of fecal contamination by starlings in cattle feed at dairy farms. A longitudinal study was conducted on 15 dairy farms in Ohio between July 2007 and October 2008. Four open-topped trays were filled with feed; two trays were placed in a food storage area and two trays were placed in an elevated area near where cattle feed. Trays were emptied monthly and discrete bird droppings were weighed for 12 consecutive months. The proportion of starling fecal samples that tested positive for *E. coli* O157:H7 and *Salmonella* was also estimated. The weights of the fecal samples collected from each farm were also standardized to adjust for variability among farms in overall levels of fecal contamination. Multilevel linear regression models with a random intercept for farm were computed to examine the association between month or season and the standardized weight of bird droppings collected each month. A total of 179 starling fecal samples were collected during the study period, and five samples (2.79%) from five farms were positive for *E. coli* O157:H7 and two samples from one farm (1.12%) were positive for *Salmonella*. The amount of contamination was significantly higher in January compared to all other months except March and December when no significant differences were observed. In addition, when months were collapsed by season fecal contamination was higher in the winter compared to all other seasons. These results indicate that the fecal contamination of the farm environment does not coincide with the period when cow fecal pats are most likely to be positive

040P (continued)
for several zoonotic pathogens including *E. coli* O157:H7. These results provide important information for future quantitative microbial risk assessments concerning the role of starlings in spreading enteric pathogens on dairy farms.

041P
Multilocus variable-number tandem repeat analysis of *Salmonella* enteritidis strains isolated in brazil and north america over a 24-year period
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Purpose: *Salmonella* Enteritidis is the most frequently isolated serotype from cases of human food-borne gastroenteritis in many countries. In the USA, the upsurge in the isolations of *S.* Enteritidis started during the 1980`s in the Northeastern States, and then spread to the west during the 1990s. In Brazil, this upsurge was observed during the 1990s with increased incidences in non-human sources. The aim of this study was to assess the genetic diversity of *S.* Enteritidis strains isolated in Brazil and North America (USA and Canada) between 1986 and 2010 by Multi-locus Variable-Number Tandem Repeat Analysis (MLVA).
Methods: A total of 288 *Salmonella* Enteritidis strains isolated in Brazil, USA and Canada from human feces (115), food (61) and poultry (112) between 1986 and 2010 were typed by MLVA. The nine loci inclusing SENTR1, SENTR2, SENTR3, SENTR4, SENTR5, SENTR6, SENTR7, SE-3 and SE-7 were amplified by multiplex-PCR followed by capillary electrophoresis. The size of the peaks were analyzed by GeneMarker (Softgenetics LLC) and the genomic similarity was assessed by constructing a dendrogram using BioNumerics (Applied Maths, Keistraat, Belgium).
Results: MLVA grouped the 288 strains from Brazil and North America into two major groups named A and B with 44% of congruence. A total of 74 strains clustered in group A which contained vast majority of strains isolated from North America (n=71) but only three pre-pandemic strains isolated from Brazil. In contrast, majority of strains isolated from Brazil (n=185) and few strains isolated from North America (n=29) were clustered in group B.
Conclusions: In general the North American strains appeared more genetically diverse. Clustering of pre-pandemic strains isolated in Brazil with North American strains suggests the possibility that these strains were more likely genetically diverse before the pandemic however, after 1993 a new and prevalent subtype of *S.* Enteritidis emerged in Brazil which has also been isolated in North America.

041aP
The frequency of detecting Shiga toxin-producing *Escherichia coli* O groups and virulence genes in feces of commercial feedlot cattle
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Purpose: Our objectives were to determine the frequency of detecting Shiga toxin-producing *Escherichia coli* (STEC) O groups (O26, O45, O103, O111, O121, O145, and O157) and major virulence genes (*stx*1, *stx*2, *eae* and *ehx*A) in fecal samples from a study that evaluated efficacy of interventions for STEC O157:H7 in over 17,000 commercial feedlot cattle.
Methods: Thirty fresh pen-floor fecal samples were collected weekly for four consecutive weeks from each of 40 pens. Samples were enriched in *E. coli* broth for six hours, DNA was extracted, and a multiplex PCR was used to detect seven serogroup-specific and four virulence genes.
Results: From 4,800 total samples, 1,273 (26.5%) were positive for one or more O group genes with samples positive for O157 (n= 701, 14.6%), O26 (n= 522, 10.5%), O103 (n= 493, 10.3%), O121 (n= 110, 2.3%), O45 (n= 88, 1.8%), O111 (n= 16, 0.3%), and O145 (n= 8, 0.2%). Overall, 27.0% (1,295/4,800) of samples were positive for Shiga toxin genes (*stx*1 and/or *stx*2) and 82.8% (1,072/1,295) of these were also positive for *eae*. Of the *stx*-positive samples, 654/1,295 were negative for all seven serogroups, while others were positive for O157 (419/1,295), O26 (290/1,295), O103 (239/1,295), O121 (82/1,295), O45 (52/1,295), O145 (6/1,295), or O111 (3/1,295).
Conclusion: Multivariable statistical analyses are still pending, yet these descriptive results provide insight on the frequency of detecting STEC serogroups and virulence genes in feces of cattle, and demonstrate that feces testing positive for Shiga toxin genes often do not contain the top seven STEC serogroups.

042P
Impact of Johne's disease, natural infection and vaccination, on bovine tuberculosis diagnostics tests
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Cross reactivity between the diagnostics tests for Johne's disease (JD) and bovine tuberculosis (BTb), particularly the impact of JD, either through natural infection or vaccination, on the BTb diagnostic tests used in national eradication programs lacks a valuable estimation. The objective of the current study was to evaluate the cross-reactivity between JD and BTb diagnostics tests using different data streams from different states in the US. From Minnesota (MN), an evaluation of the impact of JD natural infection on the caudal fold test (CFT) for BTb was performed. BTb test charts were collected from the Minnesota Board of Animal Health and Johne's disease test results from the Veterinary Diagnostic Laboratory (VDL) at the University of Minnesota, for the years 2007 through 2009. The analysis only included herds with more than 30 cattle and where a whole herd BTb testing had been performed and also had JD screening test performed within one year of the BTb test. Results shows a response rate to the CFT below 4% with no difference between JD positives and negatives. Furthermore, we also analyzed data from vaccinated herds: 6 herds from Iowa, 2 from Wisconsin and 1 from MN. These data show levels of suspect rate to the CFT from 20% to almost 50% among JD vaccinates, indicating the strong impact of vaccination for JD on the BTb diagnostic test results. This data provides valuable estimates of the impact of JD natural infection and vaccination that can drive economical and surveillance decisions at a herd level.

043P
Network analysis of cattle movements in relation to bovine tuberculosis transmission risk in Minnesota
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Bovine tuberculosis (BTb) was first diagnosed in cattle through slaughter surveillance in northwestern Minnesota (MN) in 2005. By the end of 2008, 12 cattle herds had found to be infected with BTb, and one of the causes for infection was determined to be the movement of infected animals between herds. USDA granted split-state status to MN in 2008, upgrading most of the state to modified-accredited advanced (MAA) and only a smaller area of 6,915 km2 in northwestern Minnesota as modified accredited (MA). The state has now been declared BTb free; however, since January 2008 all cattle movements within the MA were recorded electronically. The objective of this study is to characterize cattle movements in a high risk area for BTb in MN and also identify which herds might have a higher risk to become infected and to infect other herds. The data used in this analysis includes the years 2008 through

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2011. During this period, 3,467 movements were recorded with 46,717 cattle being moved, corresponding to permits issued to 559 premises, mostly representing private farms, sale yards, slaughter facilities and county or state fairs. Although, sale yards represented less than 2% of the nodes (premises), 60% of the movements were to or from a sale yard. Less than 2% of movements, both into and out of the MA zone involved locations outside MN (other states and Canada). Movements occurring between herds in the MA zone corresponded to 24% off the total number of recorded movements. Network analysis was performed on the movement data. The network showed a density of 0.4%, a fragmentation of 88% and a clustering coefficient of 14.6%. The betweenness centralization index was 12.7%. The degree distribution showed that 20% of nodes performed 90% of movements. A risk score for the private farms within the MA zone was developed in order to identify high risk herds for disease introduction.This analysis provides novel description about the contact structure of cattle movements in a high risk area for BTb, essential to support future surveillance decisions.

044P

Epidemiological analysis of BVDV infection in cattle farms of Kharkov region, Ukraine

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Purpose: This study was focused on (i) detection of BVDV specific antibodies in selected cattle farms, (ii) identification of persistently infected (PI) animals and (iii) genetic typing of selected BVDV isolates. Methods: BVDV antibodies were detected in 1023 blood samples collected from three cattle farms in the Kharkov region during period 2011-2012. A total number of animals is 815 animals in the first farm, 900 and 5500 animals in the second and the third farm. PI animals were identified by the detection of BVDV using RT-PCR employing the pan-pestivirus 324/326 primers in samples of the antibody negative cattle. Selected PCR amplicons were sequenced. Phylogenetic analysis in 5'-UTR (245 bp fragment) was used for the genetic typing of BVDV isolates into subgenotypes. Results: BVDV specific antibodies were detected in 694 of 1023 samples analyzed (67.8%). This number is in agreement with findings in many cattle herds around world. However the number of positive samples differed in the herds. While 43 samples out of 250 (17.2%) were identified in the first herd, 398 out of 473 (84.1%) and 253 out of 300 (84.3%) animals were positive in the second and the third herd. High number of animals with BVDV RNA was detected in all herds. The RT-PCR assay detected 143 of 1023 samples analyzed (14.1%). 32 samples out of 250 (12.8%) were detected in the first herd but 79 out of 473 (16.7%) and 32 out of 300 (10.7%) were found in the second and the third herd. Data on the number of PI animals were in accord with serological findings in the cattle herds involved in our study. Genetic typing of 12 isolates indicated that all viruses were typed as BVDV-1b and all of them were absolutely identical in 5'-UTR. It is not excluded that many identical isolates are the result of vaccination with the life BVDV vaccine used in those farms but this hypothesis has to be verified. Conclusions: Our results indicated that the BVDV infection is widespread in cattle herds in Kharkov region. Better characterization of viral isolates as well as the introduction of biosecurity program on the farms is in progress of Swiss-Ukraine-Slovak SCOPES project.

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045P

Comparison of *M. bovis* gamma interferon test results between tissue culture plate and microtube methods

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M. bovis gamma interferon testing (GIT) is used as a confirmatory test in cows with a positive caudal fold test. The GIT harvesting step (Plate method) involves centrifuging 24-well plates and collecting serum using a single-channel pipet, both of which are time consuming. The Microtube method stimulates lymphocytes in microtubes, rather than tissue culture plates, shortening harvesting by increasing centrifuge batch size, and allowing use of multichannel pipets. The Microtube method uses smaller sample volumes for lymphocyte stimulation, potentially producing less IFN-ɣ and false negative test results. This project compared test results between the Plate and Microtube methods of GIT.

Samples from 58 cows from 3 California dairies currently or historically infected with M. *bovis* were tested using both methods, and compared using paired t-tests. 30 of 58 cows tested positive on ≥1 GIT method, and *M. bovis* was detected postmortem in 29 of these 30 via either culture or PCR. The *M. avium*, *M. bovis*, and *M. bovis*-Nil OD values produced by the Microtube method were significantly lower than those produced by the Plate method. One sample identified as *M. bovis* positive via Plate method was negative using Microtube method.

The Microtube method produces less IFN-ɣ than the Plate method. As a result, the sensitivity of the Microtube method appears lower than that of the Plate method. Optimizing an alternative *M. bovis*-Nil threshold for the Microtube method might allow for more efficient performance of GIT without a loss of sensitivity.

046P

Reaction of the Erysipelothrix rhusiopathiae species on weeds' influence

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Bacteria of the type Erysipelothrix rhusiopathiae are well known to be causative agents of dangerous diseases to human beings as well as pets and agricultural animals. This causative agent gets into animals' organisms from earth and water, where it can live for a long time. One of the key issues in this respect is the question of the possibility to influence the density of the E. rhusiopathiae population by the multiple species of plants that are of prime importance in ecosystems.

In the series of conducted tests we aimed at investigation of weeds' metabolites Desmodesmus communis var. rectangularis (G.S.West) E.Hegewald at the density of E. rhusiopathiae cultures (VR-2 var. IVM,). The weeds have been cultivated at the Fitzgerald environment, at the temperature +22-25ºC artificial light in 25 klx (10 hours per day) during 15 days.

E. rhusiopathiae bacretia were cultivated on brain heart infusion broth (AES Chemunex) with adding 0,4 % glucose corresponding to the weight, at the temperature +36,7ºC.

For testing procedure the species of D. communis var. rectangularis weeds were filtered through depth filter Seitz EKS (Pall Corporation, Gemany). The obtained filtrate was added to the species of E. rhusiopathiae depending on its dissolving 1x10-2, 1x10-4, 1x10-6. The culture of E. rhusiopathiae was used as a means of control with adding corresponding quantities of sterile environment Fitzgerald for weeds. In 48 hours of cultivation the density of E. rhusiopathiae cells was defined both in experimental and control samples.

The results of the bacteria E. rhusiopathiae density obtained from the tests are the following - control samples 12,8±2,2x106 cells per ml, experimental samples while dissolving weeds' discharge 1x10-2 - 5,3±0,5x106 cells per ml; 1x10-4 - 7,9±0,8x106 cells per ml; 1x10-6 -10,5±1,3 x106 cells per ml. Having analysed the obtained data we can conclude that the weeds D. communis var. rectangularis discharge into water certain substances able to retard bacteria E. rhusiopathiae reproduction.

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047P

Molecular detection of Salmonella in environmental samples from meat processing facilities in Mexico

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Introduction: Disease attributed to *Salmonella* is one of the leading causes of death in children under age five in Mexico. Improper handling of products, poor hygiene and failure to maintain the cold chain are contributing factors in the prevalence of *Salmonella* in meat processing plants.
Purpose: The purpose of this study was to gather baseline information on the prevalence of *Salmonella* in meat plant environments in different parts of Mexico. This initial investigation can aid in determining the need for increased sanitation and cleanliness in the meat processing environment.
Methods: A total of 150 environmental sponge samples were collected from three different meat processing facilities in three different cities in Mexico, including Merida, Veracruz, and Playa Del Carmen. Samples were enriched using modified BPW at 36°C for 20 hours, and then aliquots of each enrichment were subjected to a PCR assay for screening. Microbiological analyses of enrichments that screened positive for *Salmonella* by the assay was continued following the procedures outlined by the US Department of Agriculture: Microbiological Laboratory Guide (USDA:MLG) for identification and isolation of *Salmonella*.
Results: Of the 150 samples analyzed, 100 tested positive for *Salmonella* by the PCR screening assay. According to microbiological analysis, the prevalence of *Salmonella* in Merida, Veracruz and Playa Del Carmen, was 76%, 60%, and 64% (56% true positive), respectively. Ninety-six percent of the PCR screen positive results were confirmed by the USDA:MLG culture method.
Significance: The results from this study have shown that there is a relatively high prevalence of *Salmonella* in Mexican meat processing plant environments. This reveals a need to improve hygiene, sanitation and control temperature in order to decrease prevalence of *Salmonella* in the processing environment to aid in the reduction of foodborne illness resulting from *Salmonella*.

048P

Development of a multiplex real-time PCR for the serotype-specific detection of *Salmonella* Enteritidis

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Purpose: Salmonella Enteritidis is a major cause of nontyphoidal salmonellosis in humans associaetd with the consumption of contaminated raw or undecoocked eggs or poultry meat. Rapid, sensitive and accurate identification of this serotype from poultry and food products is essential to ensure the safety of food products for human consumption. The aim of this study was to develop and test the sensitivity of a multiplex real-time PCR assay (TaqMan) for serotype-specific detection of S. Enteritidis isolates from eggs, environmental drag swabs and chicken meat.
Methods: The primers and probes (TaqMan) were designed to target three genes including invA (Salmonella genus-specific), sdfI (a chromosomally located S. Enteritidis-specific gene) and prot6E (a plasmid encoded S. Enteritidis-specific gene). Sensitivity test was performed using serial dilutions of the S. Enteritidis culture and DNA. The specificity of the assay was also tested on multiple strains with or without the virulence plasmid.
Results: The assay was 100% sensitive and specific, correctly identified all S. Enteritidis strains and distinguished from non-S. Enteritidis isolates. This assay also correctly distinguished S. Enteritidis plasmid and non-plasmid bearing strains. The detection limit of the assay was ≤25 CFU/reaction.
Conclusions: The multiplex real-time PCR developed in this study is efficient and fast assay for serotype-specific detection of S. Enteritidis strains. We are currently testing the efficacy of this PCR to detect S. Enteritidis in in eggs, meat and environmental drag swabs. These results will be presented and discussed.

049P

Salmonella shedding in close-up dairy heifers.

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Salmonella is a major concern for animal and public health. *Salmonella* represents a major cause of foodborne disease outbreaks. In regards to animal health, clinical salmonellosis can be costly to a dairy farm due to treatment costs, increased cull rates and reduced milk production. Previous research described a high *Salmonella* prevalence in close-up heifers. The objective of this study was to examine the *Salmonella* status of heifers before and after arrival to a close-up facility. The hypothesis was that *Salmonella* shedding increases after movement to the close-up pen. A total of 214 heifers, within three weeks of calving, were sampled two times; once in a heifer development facility and once in the close-up facility. At each sampling, 10 gram fecal samples were obtained per rectum. Fecal samples were cultured for *Salmonella* using standard methods. Presumptive *Salmonella* isolates were submitted for confirmation and serotyping. Comparison of *Salmonella* status was conducted using the McNemar statistical test. The overall proportion of heifers that were *Salmonella* positive at least once was 14.5%. One heifer was positive at both sampling points. The prevalence of positive heifers in development was 9.8%, and 5.1% were positive after arrival in the close-up facility. For the nine groups of heifers sampled, 5 had at least one positive *Salmonella* sample. Most of the positive development samples (18/21) occurred in one cohort of heifers. There was no difference in *Salmonella* prevalence between the 2 sampling points ($p>0.05$). Serotyping data is pending. These data support the previous report of high *Salmonella* shedding in heifers, and that risk factors for *Salmonella* shedding in heifers are likely multifactorial, involving temporal and movement related factors.

050P

Modeling *Salmonella* dynamics within a finishing pig farm: group structure effects on transmission

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The spatial structure of farming houses influences the spread of diseases. There is a general trend in modern, large-scale pig farming towards housing animals in larger pens, and larger grower houses. To understand the implications of this trend in the dynamics of foodborne pathogens in swine populations, we developed a spatially explicit model of *Salmonella* transmission of a grower house in an intensive, all-in-all-out system. The model is an ordinary differential equation system with four classes of individuals: susceptible, clinically infectious, subclinically infectious, and carrier. The population is divided into a discrete pen structure. Infectious pigs can transmit the infection to neighboring pens through direct contact, and to all pens through indirect transmission. Several pen configurations and sizes were evaluated through simulation. The basic reproduction number was derived for different pen configurations. Pen density and total population in the house had a larger effect on *Salmonella* prevalence than pen configuration. However, the configuration of the pens in the grower house had also significant impact on the infection prevalence. Moving the pen configuration away from a square structure and towards more linear configurations (e.g. 8×2 from 16×1) decreases the spread of *Salmonella* in the facility. The general trend of larger houses and larger pens facilitates the transmission of *Salmonella*. The physical structure of the farming facilities should not be ignored when modeling within farm transmission of foodborne pathogens.

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051P

Evaluation of Diamond V Original XPC for reducing cecal colonization by Salmonella Enteriditis in layer pullets

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Salmonella Enteriditis, the world's leading cause of human salmonellosis, is currently the focus of egg industry disease control strategies because of recent outbreaks linked with egg production and new federal regulations and oversight designed to control contamination in poultry production in the United States. In this study, we evaluated the ability of Diamond V Original XPC, a nutritional feed supplement, to determine its ability to reduce cecal colonization by *S*. Enteriditis. Two groups of day-old commercial layer pullets were fed a diet with or without XPC supplementation. Individual birds (n=20; per treatment) were challenged by oral gavage with a 10^6 CFU/ml dose of *S*. Enteriditis at 28 days of age. A third group of pullets, fed control feed and not challenged with *S*. Enteriditis, were included as a negative control treatment. Four days post-challenge, the cecal contents were obtained and enumerated to detect levels of shedding or presence/absence of the pathogen in each bird. Results of cecal count enumeration indicated a significant reduction ($P < 0.05$) of *S*. Enteriditis counts in the group fed the XPC supplement. While XPC shows potential for use as an effective control strategy, future studies conducted under commercial settings are necessary for complete evaluation.

052P

Bovine-deer-waterfowl interactions and Salmonella spp. transference

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Purpose: Salmonella is the most common foodborne illness worldwide and is capable of causing septicemia and enteritis in humans and animals, including cattle. Cattle can also be subclinically infected with Salmonella and serve as a reservoir for human infections. Reservoirs of salmonellae can also be found in various wildlife species; of particular interest are white-tailed deer and waterfowl. Deer, waterfowl and cattle often inhabit common geographic areas and have been documented to have direct and indirect contact. We hypothesized that white-tailed deer and waterfowl serve as a reservoir of Salmonella for cattle. To test this, a cross-sectional study was conducted to compare the spatial and serovar distribution of Salmonella isolated from one dairy cattle herd and the waterfowl and deer herds found on or near Kellogg Biological Station.
Methods: Free-caught fresh deer fecal pellets and waterfowl droppings were collected in the cattle pastures and surrounding areas. Both individual and pooled cattle fecal samples were collected using a proportional sampling model. The location of collection was obtained for all samples. Salmonella was cultured and serotyped using standard methods. Descriptive data, production stage (cattle), location and serovar distribution was tabulated and mapped.
Results: This study found that all species sampled tested positive for Salmonella spp., with white-tailed deer having a prevalence of 10.38%, cattle with 4% prevalence, and waterfowl with 25% prevalence. The Salmonella serovar Newport was found in all species, while the Hartford was shared by cows and waterfowl. Further serovar typing is pending.
Conclusions: This knowledge may allow for improved farm management techniques through a better understanding of the interactions between these species and the risk of both white-tailed deer and waterfowl as a reservoir of Salmonella. This knowledge will ultimately aid in the prevention of Salmonellosis in both humans and animals.

053P

Antimicrobial susceptibility of *Escherichia coli* and *Salmonella* isolated from feedlot cattle: a NARMS pilot study.

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Objective: Describe the prevalence and susceptibility of non-type specific *E. coli* and *Salmonella* across feedlots in the Texas high plains.
Methods: Convenience samples of feedlots in the Texas Panhandle were enrolled. At the initial visit to each feedlot, 4 pens were enrolled with 2 pens being within 30 days of arrival and 2 pens within 30 days of slaughter. At 3 subsequent visits, the 2 former pens were resampled whereas 2 new pens within 30 days of slaughter were selected and sampled. Within each pen, 20 pen-floor fecal samples were collected. Feedlots were visited for sample collection approximately monthly from September to December, 2011. *E. coli* and *Salmonella* were cultured from 40 g feces using standard microbial protocols. Feces were diluted (1 g into 9 ml buffered peptone water) and streaked for isolation onto CHROMagar *E. coli*. Isolates were subcultured onto MacConkey agar for further analysis. *Salmonella* detection was attained through feces enrichment (5 g into 45 ml Tetrathionate broth and Rappaport-Vassiliadis broth) then streaked for isolation onto XLT-4 agar. Three non-type specific *E. coli* isolates and up to 2 *Salmonella* isolates for each sample were collected and antimicrobial susceptibility was determined using broth microdilution.
Results: A total of 1,264 fecal samples were collected. Overall prevalence of *Salmonella* was 60.5%. Non-type specific *E.* coli was recovered from virtually all (99.8%) samples. Preliminary data show that 66.2% (764/1154) of *E. coli* were susceptible to all antibiotics tested. A further 14.1% of isolates were resistant to one antibiotic and 7.8% were resistant to 4 or more antibiotics. Of those, 25.6% and 11.1% displayed the ACSSuT and MDR-AmpC phenotypes, respectively. Isolate susceptibility within the same animal varied in 31 of 149 samples.

054P

Shedding of foodborne pathogens and microbial carcass contamination of hunted wild ruminants

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Purpose: Because healthy game animals have basically the potential to carry zoonotic pathogens and the microbial status of carcasses is influenced by highly variable factors, the aims of this study on hunted wild ruminants were to assess the shedding of selected bacterial foodborne pathogens and the microbial contamination of carcasses. Methods: Fecal samples from hunted wild red deer (n = 84), roe deer (n = 64), chamois (n = 64), and ibex (n = 27) were examined for *Salmonella* spp. (ISO 6579:09.2006), *Listeria monocytogenes* (ISO 11290-1:2004), and *Escherichia coli* harboring *stx* encoding Shiga toxins and *eae* encoding intimin (real-time PCR after enrichment in mTSB with novobiocin). Isolated strains of Shiga toxin-producing *Escherichia coli* (STEC) were tested for *stx*1, *stx*2, and *eae.* In addition, surfaces of 328 skinned carcasses from 136 red deer, 122 roe deer, and 70 chamois were examined for total viable counts and *Enterobacteriaceae* by swabbing. Results: All 239 fecal samples tested negative for *Salmonella* spp. and *Listeria monocytogenes* but other *Listeria* species were found in 11 (4.6%) samples. Besides, 78 (32.6%) samples tested positive for *stx*, 16 (6.7%) for *eae* and 33 (13.8%) for both *stx* and *eae*. Among the 56 isolated STEC strains, 67.8% were positive for *stx*2 (alone or in combination with *stx*1). The distribution of *stx*1 and *stx*2 genes differed between the examined animal species. Only two STEC strains harbored *eae*. The investigation of skinned carcasses showed that average total viable counts (4.0-4.2 log CFU cm-2) and *Enterobacteriaceae* counts/detection rates (2.3-2.6 log CFU cm-2; 87.5-90%) were comparable for the examined animal species, but differed by several orders of magnitude between certain abattoirs. Conclusions: Wild ruminants (red deer, roe deer, chamois, ibex)

054P (continued)
constitute a reservoir for STEC, but further characterization of the isolated strains is required to assess their actual human pathogenicity. Strict compliance with good hunting and hygiene practices during every step is of great importance to avoid contaminations and to prevent foodborne pathogens from entering the food chain.

055P
Frequency of Escherichia coli O157:H7 SNP genotypes in different cattle production systems, seasons, and sample types
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Purpose: Escherichia coli O157:H7, a zoonotic human pathogen for which cattle are a frequent reservoir host, includes several genotypes that differ in apparent virulence. The purpose of this study was to determine the distribution of these genotypes among isolates obtained from different cattle production systems, at different seasons, and from different sample types.
Methods: A 48-plex single nucleotide polymorphism (SNP) typing system using the Illumina GoldenGate platform was developed and utilized to classify >600 E. coli O157:H7 isolates from cattle farms into twelve clades. The distribution of these clades among different isolate sources was examined.
Results: Some clades were over-represented among isolates from human infection (clinical genotypes, CG) while others were frequently isolated from cattle but uncommon in human infection (bovine-biased genotypes, BBG). Based on logistic regression models, the odds of CG were significantly higher for feedlot isolates than for dairy isolates in both summer and winter (ORs =11.3 [95% CI 6.5-19.7] and 2.2 [95% CI 1-5.1], respectively). Among both dairy and feedlot isolates, the odds of CG were higher for summer isolates than for winter isolates, but this difference was only statistically significant among feedlot isolates (OR = 6.5 [95% CI 2.8-15.4]). The odds of CG were significantly higher for water isolates than for fecal isolates on dairies (OR = 3 [95% CI 1.4-6.4]). Among feedlot isolates, the odds of CG were higher for fecal isolates than for water isolates although the difference was not significant. No significant difference in the odds of CG was observed for fecal versus recto-anal junction isolates.
Conclusions: Clinical genotypes of E. coli O157:H7 are strongly associated with feedlot cattle production systems. Understanding the basis of this association could lead to development of novel management strategies to promote food safety and public health.

057P
Prevalence and characterisation of CTX-M beta-lactamases amongst ExPEC from humans, companion animals, food-producing animals and retail meats in China
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Purpose: To investigate the resistance of ExPEC producing extended-spectrum beta-lactamases (ESBLs) in China.
Methods: A total of 1,956 E. coli isolates recovered from humans, companion animals, food-producing animals and retail meats were submitted for examination for ExPEC by using multiplex PCR. MICs of 16 antibiotics against ExPEC strains were determined by agar dilution method, then double disk synergy test was conducted to screen for ESBL-producing. Multiplex PCR, DNA sequencing and PFGE were used to determine the CTX-M genotype and clonal subtype. Conjugation experiments were also carried out and the replicon types of plasmids were analyzed.
Results: Approximately 13.4% (262 of 1,965) of the E. coli isolates were identified as ExPEC. The occurrence of ExPEC was highest in E. coli isolated from companion animals (30.8%) and humans (23.4%), and less frequent in isolates from food-producing animals (7.1%) and retail meats (5.4%). Among the 16 antimicrobial agents tested, resistance to ampicillin (82.0%), nalidixic acid (79.0%), tetracycline (77.9%), and sulfamethoxazole (75.2%) was most frequent. 84 (32.1%) ExPEC isolates exhibit an ESBL phenotype. 34.5% (29) were CTX-M-14, 22.6% (19) were CTX-M-55, 11.9% (10) were CTX-M-15 and 10.7% (9) were CTX-M-65. PCR mapping and sequencing of representative products revealed six types of blaCTX-M-1G genetic environment, denoted 1G-I to 1G-VI and nine types for blaCTX-M-9G genes, denoted 9G-I to 9G-IX. IncFII, IncFIB, IncFIA, IncI1, IncN and IncB/O replicons were detected in 23, 13, 9, 9, 5 and 3 of the 50 transconjugants carrying blaCTX-M, respectively. 75.0% of the plasmids encoding CTX-M-15 were F31:A4:B1 plasmids and 21.1% and 21.1% of the plasmids encoding CTX-M-14 were F2:A1:B1 and F35: A-:B- plasmids, while F18:A-:B1 and F33:A-:B- were common among the plasmids encoding CTX-M-55 (18.2% was identified, separately).
Conclusions: This study demonstrates that ExPEC from animals can be important reservoirs of blaCTX-M genes and may contribute to the dissemination and transfer of these beta-lactamase genes throughout China.

058P
The prevalence, and characterization of shiga-toxin producing *escherichia coli* (stec) serotypes from feedlot and range cattle in the us midwest.
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Since the emergency of Shiga toxin-producing *Escherichia coli* (STEC)
strains in 1970s as important foodborne pathogens *E. coli* O157:H7 has been the most frequently isolated STEC serotype associated with severe e illness including Hemolytic Uremic syndrome. However, lately, non-O157 serotypes have caused serious outbreaks as well prompting the USDA, FSIS to publish the intent to regulate the presence of STEC belonging to serogroups O26, O45, O103, O111, O121, and O145 in non-intact beef products. This study determined the prevalence and characterization of STEC serotypes shed in feces of Feedlot and Range Cattle in ND. The *E. coli* isolates were tested in a four primer multiplex PCR assay for detection and amplification of the Shiga-toxin like genes for *E. coli* O157:H7 (*stx*1 and *stx*2). All 204 isolates that were positive for *stx* genes were serotyped using multiplex PCR targeting the *wzx* (O-antigen-flippase0 - of O26, O45, O103, O111, O113, O121,- O145, and O157 serogroups. Overall the prevalence of STEC serotypes was; O26 (53/204)7%; O103 (46/204)11%; O111 (55/204)8%; O145 49/204)13%. Prevalence in calves was; O26 (13/57)23%; O103 (12/57)21%; O111 (16/57)28%; O145 (15/57)26%. Prevalence in Adults was; O26 (40/147)27.2%; O103(34/147)23.1%; O111(39/147)26.5%; O145 (34/147)23.1%.The widespread distribution of STEC serotypes O26, O45, O103, O111, and O145 and the detection of *stx* 1, *stx* 2, raises public health concerns. These data provide further evidence of the widespread shedding of non-o157:H7 STEC in feces of feedlot and ranch cattle in ND.

059P
A Meta-analysis of the association of *Lactobacillus acidophilus* NP51 administration with *Escherichia coli* O157 in feces and on hides of feedlot cattle.
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Introduction: A growing body of evidence suggests that pre-harvest control of *Escherichia coli* O157 will likely positively impact human health. Inclusion of the direct-fed microbial *L. acidophilus* NP51, in feedlot rations has been associated with decreased burden of *E. coli* O157 in feces and on hides of cattle.

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059P (continued)
Objectives: To a) assemble available data from studies that have evaluated an association of *L. acidophilus* NP51 with *E. coli* O157; b) reanalyze the data using harmonized models; and c) perform a meta-analysis to produce a summary effect measure and evaluate between study variance.
Methods: Pen-level fecal and hide prevalence data were gathered from 16 trials that administered *L. acidophilus* NP51 at10^9 CFU/animal/day, 10^7 CFU/ animal/day, or both. Complete fecal and hide data were available for16 and 9 studies, respectively. Data will be analyzed to produce study-level relative risk estimates (and their 95%confidence intervals) using generalized linear mixed models. The inverse of study-level variance will be used to weight each observation and study-to-study variance will be assessed and if significant, meta-regression will be performed to evaluate potential variables (e.g., dose) that may explain variation. Outcomes of interest include post-exposure measure of effect, terminal measure of effect, and a dose response.
Results: Data were identified from 16 studies. A preliminary meta-analysis of a subset of data identified an approximately 50% and 40% reduced likelihood of recovering *E. coli* O157 from feces and hides, respectively, of cattle administered *L. acidophilus* NP51 compared to control animals. More complete analyses will be presented.
Conclusion: Meta-analysis is a valuable tool to evaluate between-study variation and produce a summary measure of effect. However, variability in design, data presentation, and a paucity of consistently measured covariates can add substantial challenges to meta-analysis.

GASTROENTERIC DISEASES POSTERS

060P
Case-control assessment of microbiological etiology associated with calf diarrhea in Midwest USA
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Calf diarrhea is a major economic burden for the US cattle industry. A variety of infectious agents are implicated in calf diarrhea and co-infection of multiple pathogens is not uncommon in diarrheic calves. A case-control study was conducted to assess infectious etiologies associated with calf diarrhea in Midwest cattle farms. A total of 199 and 245 fecal samples were obtained from diarrheic and healthy calves, respectively, from 165 cattle farms. Samples were tested by a panel of multiplex PCR assays for 11 enteric pathogens: bovine rotavirus group A (BRV-A), bovine coronavirus (BCoV), bovine viral diarrhea virus (BVDV), bovine enterovirus (BEV), bovine norovirus (BNoV), Nebovirus, bovine torovirus (BToV), *Salmonella spp. (Salmonella)*, *Escherichia coli* (*E. coli*) $K99^+$, *Clostridium perfringens* with β toxin gene and *Cryptosporidium parvum* (*C. parvum*). The association between diarrhea and detection of each pathogen was analyzed using a multivariate logistic regression model. More than a half of the fecal samples from the diarrheic calves had multiple pathogens. Statistically, BRV-A, BCoV, BNoV, Nebovirus, *Salmonella*, *E. coli* $K99^+$, and *C. parvum* were significantly associated with calf diarrhea ($p<0.05$). Among them, *C. parvum* and BRV-A were considered to be the most common enteric pathogens for calf diarrhea with high detection frequency (33.7% and 27.1%) and strong odds ratio (173 and 79.9). Unexpectedly BNoV (OR=2.0) and Nebovirus (OR=16.7) were identified with high frequency in diarrheic calves, suggesting these viruses may have a significant contribution to calf diarrhea.

061P
Phenotype array comparison of highly divergent *Clostridium difficile* strains
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One of the contributing factors to increased rate of infection is the emergence of hypervirulent strains of *C. difficile* and the high genome diversity in this species. Since the core genome of *C. difficile* is composed of less than 25% of the pan-genome, the impact of this high genome diversity on this species phenotype has not been explored. Therefore, from a collection of 100 *C. difficile* strains, 6 isolates that belonged to different year of isolation, geographical location and varying genome diversity were screened for the complete phenotype profile. The complete phenotype profile of the strains was obtained by screening against Biolog phenotype microarray (PM) panels 1 through 20. For PM 1through 8, 50% increase in signal intensity over negative control was considered positive and for PM 9 through 20, 100% increase from the lowest signal for each PM was considered non-sensitive. The PM analysis revealed that despite high genome diversity, most phenotypes had a similar profile for the strains compared. However, we also find that some of the strains do have expanded metabolic profile. For example the strain QCD23m63 was not able to utilize ethanolamine as nitrogen source while strain CD630 was able to utilize ethanolamine. We further analyzed the correlation of this phenotype differences by whole genome comparisons of the strains involved. We find that while strain CD630 harbors a cluster 21 genes that are involved in ethanolamine utilization pathway, the strain QCD23m63 lack this whole cluster. Therefore our phenotype screening results is in agreement with genome comparison results. Ethanolamine is an abundant nitrogen source in gastrointestinal tract of animals and strains that could utilize this compound as nitrogen source therefore will have a selective growth advantage and colonization potential. Our results therefore demonstrates the power of combining high-throughput phenotype screening with genome scale comparisons to unravel phenotypic traits that are otherwise not revealed by traditional screening methods.

062P
Establishment of transcriptome landscape of multiple Clostridium difficile strains using RNA sequencing
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Clostridium difficile (CD) is an emerging spore forming enteric bacterium. In the past two decades the rate of C. difficile infection (CDI) has been steadily on the rise. The emergence of a hypervirulent strain type that belongs to PCR ribotype 027 has been linked to this high CDI incidences in the recent years. Comparative genomic analysis of several CD isolates from Europe and North America has revealed very high genome variability in this species and this genome plasticity has been shown to contribute to the increased virulence. In order to understand the increased virulence properties of newly emerged strains, using mRNA sequencing (RNA-Seq) we have performed in vitro transcriptomic analysis of four outbreak and non-outbreak associated hypervirulent and non-hypervirulent strains grown under in vitro conditions. Growth in Brain heart infusion medium, minimal defined medium and osmotic shock were used as in vitro conditions for comparing these strains. 20µg of total RNA isolated from these cultures were depleted of ribosomal RNA. 200ng of this depleted RNA was used for the preparation of sequencing library. Paired end 100 base RNA-seq was performed in an Illumina Hiseq 2000 machine. Paired end reads were aligned to the respective reference genome using TopHat program. TopHat alignments were generated in SAM/BAM format and further alignment manipulation was performed with SAMTools v. 0.1.7 and custom perl scripts. Transcript expression levels were then estimated as Fragments Per Kilobase per Million mapped reads (FPKM), using cufflinks program for transcript assembly and quantitation. For each strain, we find that more than 95% of genes were expressed under at least one of the conditions tested. Several hypothetical proteins, response regulators, cell surface proteins and transporters were among the differentially expressed proteins. This is the first report where transcriptomes of multiple CD strains are

062P (continued)
compared using RNA-seq. The differentially expressed genes indentified in this study could be candidates for further characterization using gene knockouts. and could lead to a better understanding about the mechanisms behind the emergence of hypervirulent CD strains.

063P
Proteomic comparison of historic and recently emerged hypervirulent Clostridium difficile strains
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The impact of Clostridium difficile infection (CDI) on healthcare is increasingly being recognized as it represents the most common infectious cause of nosocomial diarrhea. A rising number of CDI cases and outbreaks have been reported worldwide. This increase in CDI in the last two decades has been attributed to the emergence of new hypervirulent C. difficile (CD) strains. Massive variation among the genomes of CD strains I one of the reasons behind the the enhanced virulence of these new strains. Here, we compare the proteomes of five historic and newly emerged CD strains using semi-quantitative analysis of differential proteomes following in vitro incubation using isobaric tags for relative and absolute quantification (iTRAQ). We have used growth in Brain heart infusion, minimal defined medium and osmotic shock as three physiologically relevant in vitro conditions. Proteins retrieved from the in vitro cultures were subjected to in-solution digestion, iTRAQ labeling, two-dimensional liquid chromatographic tandem mass spectrometry and statistical analyses. More than 700 distinct proteins were identified in each of the strains compared in this study and were available for quantitative measures in both biological and technical replicates. Several hypothetical proteins, response regulators, cell surface proteins and transporters were among the differentially expressed proteins. With analyses of cluster of orthologous group and protein-protein network interaction, we identified the proteins that might play roles in adaptive response to the general environment, hence enhancing pathogenicity during CDI. This report represents the first documented comparative differential proteome analysis of historic and newly emerged C. difficile strains and identifies proteins that are highly modulated in vitro. The proteins could be potential candidates for diagnostic or therapeutic measures against CDI.

064P
Immune response and protective efficacy of live attenuated Salmonella vaccine expressing antigens of Mycobacterium avium subsp. paratuberculosis against challenge in mice
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Mycobacterium avium subsp. paratuberculosis (MAP) causes chronic granulomatous enteritis in ruminants that leads to diarrhea and eventually death. Existing vaccines are ineffective as they can only delay the onset of symptoms but do not protect against infection. To develop an effective vaccine, we constructed an attenuated Salmonella (Δ yejE; Δ ssaV) strain harboring a plasmid that expressed a fusion protein comprised of the Salmonella Type III secretion system (T3SS) effector SopE and MAP antigens (85A, 85B, SOD, 74F). Of various SopE-MAP fusion proteins analyzed, only SopE104-Ag85A C-terminal202-347-SOD N-terminal1-72-Ag85B C-terminal173-330 and SopE104-74F1-148+669-786were successfully expressed and secreted into culture media as revealed by western blot analysis. Mice immunized with attenuated Salmonella (Δ yejE; Δ ssaV) harboring the SopE104-Ag85A C-terminal202-347-SOD N-terminal1-72-Ag85B C-terminal173-330 and SopE104-74F1-148+669-786plasmid generated a potent and long lasting Th1 response characterized by production of IFN-γ. The cytokine profile varied at various time points after immunization and challenge, which showed down regulation of Th2 cytokines (IL-4, IL-10) and up-regulation of proinflammatory cytokines (IL-12 and IL-17). Further, the immune response correlated with protection as revealed by reduced bacterial load and improved histopathology of spleen and liver, which showed fewer granulomas and lower numbers of acid-fast bacilli as compared to PBS controls. Interestingly, vaccination with antigens mixed with Ribi adjuvant (Agmix+Ribi) imparted better protection than the attenuated salmonella vectored vaccine. Thus, priming with a live recombinant Salmonella strain that secretes MAP antigens represents a promising approach that could lead to development of an efficacious and cost effective vaccine for Johne's disease.

065P
Potential new novel *in vitro* model for long term study of *Mycobacterium avium* ssp. *paratuberculosis* infections
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M. avium spp. *paratuberculosis* (MAP) is a slow growing organism that is the causative agent of Johne's disease (JD) in ruminants and has long been suggested to be associated with Crohn's disease (CD) in humans, however this is still controversial. Although there is no direct evidence that MAP is the primary etiological agent for CD, most CD patients are found to have MAP in their intestinal tissues. JD is a contagious, chronic, and sometimes fatal infection that primarily infects the small intestines of ruminants. In cattle, the main symptoms are persistent diarrhea and wasting. These symptoms usually do not show until about 3 years of age. If by then they do not show any signs of sickness, they are usually immune. In humans, CD is considered an autoimmune inflammatory disease that can affect any part of the gastrointestinal (GI) tract. This disease can cause a wide variety of symptoms, caused by the immune system attacking the GI tract and producing inflammation. There is currently no effective cure for JD or CD; however there are medications to treat the symptoms and bacterial infections. There is no cure for MAP infections and there is no good *in vivo* mouse model. When mice are infected with MAP, they do not show the same symptoms and GI lesions that would normally occur in ruminants. Without a good mouse model, it is hard to monitor MAP infections and test novel new therapeutics. This is an issue, as testing in ruminants can be quite costly and timely. A previous study showed that phorbyl myristate acetate increased the update of *M. tuberculosis in vitro*, and vitamin A and vitamin D extended the lifespan of the cells. We found their findings to be interesting, and wanted to see if the same could be done in J774A.1 murine macrophages. This cell line has a very short lifespan of about 4-6 days. We were able to extend the lifespan of these cells to an optimal 45 days. This allowed us to monitor chronic infection *in vitro*, as well as test drugs and their efficacy. This new insight into a potential new novel *in vitro* model could potentially eliminate blind testing in mice and ruminants, and give the researcher insight into new drug therapeutics before actually embarking on *in vivo* testing.

066P
Adhesion to and invasion of bovine and human colonic epithelial cells by non-O157 Shiga toxin-producing Escherichia coli
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Assays were conducted to determine the ability of representative strains of non-O157 Shiga toxin-producing *E. coli* (STEC) to adhere to and cause attaching-effacing or other lesions in bovine and human colonic mucosal epithelial cells. This work is needed to provide a basis for development of pre-harvest interventions for those non-O157 STEC that cause the greatest risk of human infection.
Mucosal explants and primary cell cultures from the colons of slaughtered cattle were inoculated with 13 representative strains divided among STEC serogroups O26, O45, O103, O104, O111, O121, and O145. Duplicate experiments were done with human Caco-2 cells. Adherence in explants was evaluated by standard light microscopy of tissue sections stained by immunohistochemistry (IHC), and by scanning electron microscopy (SEM).

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066P (continued)
Adherence on Caco-2 cells and primary bovine colonic cells was evaluated by IHC, fluorescent actin staining with confocal microscopy, and SEM. Quantitative adherence data was limited to assays using Caco-2 cells and primary cells. Strains that showed morphological evidence of invasion were further tested by standard invasion assays using Caco-2 cells. Based on IHC, 11 of 13 strains exhibited adherence to explants. Non-inoculated explants and explants inoculated with non-pathogenic *E. coli* showed no adherent bacteria. Invasion with intracellular epithelial replication in explants was detected with one O103 and one O104 isolate. The same O103 isolate also demonstrated evidence of invasive ability in Caco-2 cells. Preliminary studies have been conducted to test for adherence using Caco-2 cells and primary cells. Non-O157 STEC commonly adhere to colonic epithelium of cattle. Of the strains tested, 85% showed adherence to explants. Some STEC also are invasive in colonic epithelial cells. Further studies are needed to determine the mechanisms of STEC adherence and invasion in the bovine intestine.

067P
Targeting *Salmonella* essential genes with antisense peptide nucleic acid
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Salmonella is capable of infecting both human and animal hosts and remains the leading cause of extraintestinal focal infection in developing and developed countries. Surveillance of the bacteria over the past 50 years has reported a sustained proliferation in the incidence of multidrug resistant strains and these alarming characteristics provide great incentive to develop a novel antibacterial agent that can effectively mitigate *Salmonella* infections. Peptide Nucleic Acids (PNA) are oligonucleotide analogs that can be engineered as antisense agents capable of silencing specific essential genes required for bacterial viability. This approach hinges on the bioavailability of the PNA inside the bacteria. Oligonucleotide entry into the cell is barred by the cellular membrane but this barrier can be surmounted by introducing a cell penetrating peptide (CPP) that mediates transmembrane transport of the oligonucleotide. We investigated the capability of antisense peptide nucleic acids conjugated to the $(KFF)_3K$ cell penetrating peptide to target possible essential genes (ligA, rpoA, rpoD, engA, tsf, and kdtA) in *Salmonella enterica* serovar Typhimurium. In order to silence the critical target genes, PNA constructs were chosen to be complementary to a specific region of the critical genes' mRNA including the translation start codon and the 5' terminal region. Antibacterial activity of all PNA-CPP conjugates was evaluated both *in vitro* and in cell culture. All PNA-CPP conjugates demonstrated some level of antimicrobial activity in a sequence-specific and dose dependent manner at micromolar concentrations. The greatest efficacy was observed from the anti-rpoA and anti-rpoD PNA-CPP conjugates, both of which were able to clear infection *in vitro* and significantly reduce infection in cell culture. The novelty and effectiveness of antisense PNAs suggests that PNA constructs represent a viable method to challenge and mitigate *Salmonella* infections. Moreover, this basic mechanism of using PNA constructs to target critical genes could conceivably challenge any bacterial strain and may play a role in addressing the need for innovative therapeutics to combat antibiotic resistant strains.

068P
The iron-sulfur protein Cj0369c contributes to the aerotolerance of Campylobacter jejuni
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Purpose: Campylobacter jejuni is a major foodborne pathogen. As a microaerophilic bacterium, C. jejuni is highly sensitive to atmospheric oxygen, which inevitably generates reactive oxygen species causing severe damage to cells. To detoxify reactive oxygen species, C. jejuni has to utilize various oxidative stress defense systems. A gene encoding a putative 2[4Fe-4S] ferredoxin (Cj0369c) was identified upstream of, and co transcribed with the pleiotropic regulator CmeR. This class of proteins is important for redox sensing and balance and play critical roles in bacterial physiology. In this study, we analyzed the role of Cj0369c in the aerotolerance and oxidative stress defense.
Methods: The cj0369c gene was mutated and complemented in C. jejuni. The susceptibility to three different oxidants was tested by disk diffusion assay. The MICs of various antimicrobials were determined with a microtitre broth dilution method. To examine if Cj0369c affects the susceptibility of C. jejuni to oxygen tension, Cj0369c mutant was compared with the wild-type and complemented strain for growth under aerobic (normal atmosphere) and microaerobic conditions.
Results: Resistance to all the oxidants did not differ between wild-type C. jejuni 11168 and Cj0369c mutant. Our results also did not reveal any differences between the mutant and the wild type in their susceptibility to the tested antibiotics. Compared to the wild-type strain, the Cj0369c mutant shows no differences in growth under microaerobic conditions. However, the Cj0369c mutant showed a 3-log reduction in viability compared with the wild type when incubated under aerobic conditions. Complementation of the mutant in trans with a plasmid-carried Cj0369c partially restored its tolerance to normal atmosphere.
Conclusions: This study suggests that Cj0369c contributes to oxygen tolerance in Campylobacter. However, we did not observe any reduction in oxidants stress resistance of Cj0369c mutant. A similar result was also reported for another aerotolerance related ferredoxin FdxA in C. jejuni, which suggested the unique role of ferredoxin in atmosphere oxygen defense among this microaerophilic pathogen.

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069P
Effects on lymphocyte subpopulations of ionized alkali mineral complex-containing diets in porcine reproductive and respiratory syndrome virus infected pigs.
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Porcine reproductive and respiratory syndrome (PRRS) is one of the most significant swine diseases worldwide. Current vaccination strategies only provide a limited protective efficacy. There are many investigations of the interaction and main effects of mineral supplements on growth and immune response in animals. Recently, Barodon (Barodon-S.F., Ansung, Gyeonggi, Korea), the anionic alkali mineral complex solution containing silica, sodium, silver, and potassium ion, was developed as a feed additives for animals. The effect of Barodon-containing feed on immune response to PRRS virus vaccination and infection in pigs was investigated. A total of 40 pigs were divided into 8 treatment groups. Four groups of pigs were vaccinated at 3 weeks old. For seven weeks, all pigs were fed with the experimental diets containing 0% (control), 0.025%, 0.05% and 0.1% of BARODON. All pigs were challenged with PRRS virus at 6 weeks old. The proportion of leukocyte subpopulations among peripheral blood was analyzed by flow cytometry. Cytotoxic T cell (CD3+CD4-CD8+) proportion was not influenced by vaccination and mineral feeding. Memory T helper cell (CD3+CD4+CD8+) proportion, on the other hand, was significantly higher in vaccinated groups than non-vaccinated groups at 6, 8 and 10 weeks old. Among the vaccinated groups, mineral fed groups showed significantly higher memory T helper cell ratio than the control group. B cell (CD3-CD21+) ratio was decreased at 2 weeks after the challenging in non-vaccinated groups but was maintained in vaccinated groups. B cell ratio of 0.05% and 0.1% Barodon-containing diet fed groups were higher than others at 6 weeks old regardless of the vaccination. PRRS virus vaccination was associated to increase of memory T helper cell ratio and to maintain of B cell ratio in PRRS virus infected pigs and those immune response was enhanced by feeding with anionic alkali mineral complex, Barodon.

IMMUNOLOGY POSTERS

070P

Porcine macrophage Cdelta2+ and Cdelta2- cell lines support influenza virus infection and replication and Cdelta2+ cells mount innate immune responses to influenza virus infection.

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Respiratory epithelial cells are the first cells which are infected with influenza virus and these cells play a major role in influenza pathogenesis. However, many studies have shown that alveolar macrophages also play a very important role in the pathogenesis and immunity to influenza infection. Until recently, useful porcine macrophage cell lines were not available for viral/influenza studies. Recently two monocyte-derived porcine macrophage cell lines (Cdelta2+ and Cdelta2-) have been developed and characterized for their phagocytic ability and biochemical properties. We further characterized these two cell lines for the presence of sialic acid based cell surface receptors responsible for the influenza virus infection. Both Cdelta2+ and Cdelta2- cells mainly expressed sialic acid receptor Sial-2,6-Gal as determined by SNA lectin binding using flow cytometry. The next step was to check if influenza viruses (swine H1N1/A/SW/IOWA and human B/FLORIDA/4/2006 strains) were able to infect and replicate in these cells. Using immunoflourescence assays, we were able to show that these viral strains infected both cell lines. We also determined the percentage of Cdelta2+ and Cdelta2- cells which were infected by both of these viruses using flow cytometry. We were also able to show, using hemagglutination (HA) assays, that both influenza A and B viruses were able to replicate in both Cdelta2+ and Cdelta2- cells. We further infected Cdelta2+ cells with these two influenza viruses and studied the changes in gene expression of different pathogen-recognition receptors (PRRs), cytokines, chemokines and anti-microbial peptides. Both swine influenza A virus and human influenza B virus strains induced significant changes in various toll-like receptors (TLRs), RIG1-like receptors (RLRs), type 1 interferons, pro-inflammatory cytokines and chemokines gene expression. Overall, we showed that both Cdelta2+ and Cdelta2- cell lines are susceptible to influenza infection and can be successfully used to study the pathogenesis of swine influenza viruses.

071P

Serological surveillance of vesicular stomatitis and swine vesicular disease in korea

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Purpose :Foot and mouth disease (FMD), swine vesicular disease (SVD), classical swine fever (CSF), vesicular stomatitis (VS) and African swine fever (ASF) are major OIE listed diseases which affect swine. Among them, SVD, VS and ASF have never occurred in Korea. In this study, statiscally designed serological surveillance had been conducted annually from 2004 to 2011 to provide sufficient evidences of SVD and VS free status in domestic pig populations in Korea.
Methods : Surveillance model was designed by considering the appropriate sampling strategy, characteristics of the disease, stratification, diagnostic method, calculation method and the surveillance procedure. Sera were analyzed by enzyme-linked immunosorbent assays (ELISA) which are recommended as one of prescribed or alternative serological tests for SVD and VS.
Results :A total of 13,676 samples from 2,154 farms were collected and tested by ELISA and were all shown to be negative for antibodies to SVD. A total of 10,275 samples from 1,616 farms and 10,509 samples from 1,637 farms were collected and tested by NJ-ELISA and IND-ELISA respectively and were all shown to be negative for antibodies to VS.
Colclusions : In conclusion, our data of 8 years show that domestic pig populations in Korea are free of SVDV and VSV infections.

072P

Development of an epitope-based vaccine against swine influenza A virus using *Escherichia coli* heat-labile toxin B subunit as a carrier-adjuvant

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Influenza A virus causes a highly contagious respiratory disease in a variety of avian and mammalian hosts, including humans and pigs. The primary means for preventing influenza epidemics is vaccination. However, the efficacy of vaccination for influenza virus is limited by frequent antigenic variations of the virus glycoproteins. In order to obtain a broadly effective vaccine against the various strains of the virus, the objective of this study was to evaluate the immunogenicity of an epitope-based vaccine candidate comprising a set of consensus epitopes among H1N1 influenza A viruses. To enhance the immunogenicity of the epitope-based vaccine, a subunit of the bacterial heat-labile enterotoxin (LTB) was used to construct a LTB-SIVe fusion antigen. The potential application of this LTB-SIVe antigen in influenza vaccine development was determined in a pig model. Pigs were immunized with the LTB-SIVe, and challenged with a pathogenic swine influenza virus, A/Sw/Iowa/40766/1992 (H1N1, α-clade). The LTB-SIVe induced antigen-specific IgA and IgG responses in serum and IgG responses in mucosal secretions. The expression of various cytokines from peripheral blood mononuclear cells was measured at 5 days post-challenge, and the result showed that IL-1β, IL-8 andIL4 expression was up-regulated in vaccinated pigs. In comparison to the non-vaccinated pigs, vaccinated pigs showed improved protection against the H1N1 influenza virus challenge, with significant reduction in virus-induced fever and pneumonic lesions. In addition, significant reduction of the viral load in nasal secretion and ileum was observed. This study established a model system for future construction of epitope-based vaccines against influenza A virus infection.

073P

Impact of oral meloxicam on circulating physiological parameters in beef steers after long distance transportation

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Bovine respiratory disease (BRD) is a serious consequence of transportation stress, resulting in significant economic losses to producers due to increased mortality, decreased animal productivity, and increased labor and medication costs. Meloxicam provides pain relief and anti-inflammatory effects in cattle for several days after a single oral treatment. Our hypothesis was that meloxicam administration before shipping will mitigate circulating physiological parameters in beef steers after long distance transportation. 97 beef steers enrolled in the Tri-county Steer Carcass Futurity (TCSCF) Cooperative In Tabor, Iowa were sourced from the Brown Loam Experiment Station in Mississippi. Calves were blood sampled to test for biomarker determination and then randomly assigned to receive either 1 mg/kg meloxicam (n=47) or a lactose placebo (n=48) orally prior to transportation. Calves were then shipped for 1,316 km to the feedlot in Tabor, IA where they were blood sampled on arrival and 6 days later. Changes in plasma proteins, TCO2, fibrinogen and hematology data between treatment groups over time were compared using a Mixed Effects Model with statistical significance designated as $P < 0.05$.

073P (continud)
There was a significant difference in neutrophil, lymphocyte, platelet, monocyte, white blood cell and red blood cell count over time ($P < 0.001$). There was an effect of treatment on circulating white blood cell count ($P=0.0194$) and evidence of a time by treatment interaction on monocyte count ($P=0.0477$). Circulating monocyte count was significantly higher in the placebo-treated control calves compared with meloxicam-treated calves immediately after transportation ($P=0.013$). No other treatment effects were observed. The results suggest that meloxicam administration may reduce the impact of transportation on circulating physiological parameters in beef calves.

074P
Comparison of P2X7 receptor antagonists with bovine cells
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Recent research has implicated extracellular ATP as a possible mediator of inflammation in several tissues and organs, including the lung. Of the various nucleotide receptors found on the cell surface, $P2X_7$ is likely the one that mediates most of a cell's response to extracellular ATP. Unfortunately, many of the antagonists previously used to block this receptor are not specific, and are known to interact with several other purinergic receptors besides $P2X_7$. Newer $P2X_7$ receptor antagonists have been released that are very specific towards the human $P2X_7$ receptor, but their applicability and function against the bovine $P2X_7$ receptor had not been tested. In the present study, we performed a side-by-side comparison of two newer receptor antagonists, KN62, and A438079, along with two older receptor antagonists, periodate-oxidized ATP (oATP) and Coomassie brilliant blue G (BBG). Using bovine Mac-T cells, we examined permeability changes in cell monolayers, calcium influx into the cell cytoplasm, and movement of a large fluorescent marker, Yo-Pro, into the cells after ATP stimulation. All 4 antagonists were equal in their ability to reduce monolayer permeability changes associated with exposure to 1 µM ATP. ATP stimulation of cells did not lead to the influx of calcium into the cell cytoplasm, regardless of whether or not additional calcium was added the extracellular environment or higher concentrations of ATP were added. In conclusion, it appears the newer antagonists have similar ability to block $P2X_7$ interactions by ATP when compared to the older antagonists.

075P
Staphylococcus aureus inhibition of dendritic cell apoptosis
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Mastitis, an inflammation of the mammary gland, costs the dairy industry over $ 2 billion annually. Staphylococcus aureus causes chronic mastitis that is difficult to treat with current therapeutics, specifically antibiotics. The goal of this study is to elucidate the mechanism by which S. aureus infects mammary dendritic cells (DC), and evades immune responses and antibiotic treatments. One such mechanism may be the prevention of apoptosis, a programmed form of cell death, by S. aureus following infection of DC. Inhibiting apoptosis in DC would benefit the pathogen by providing a replicative niche, an escape from treatments, and a reprieve from the immune system. Bovine peripheral blood mononuclear cells were isolated from whole blood with a ficoll-paque gradient. The mononuclear cells were differentiated into DC using Granulocyte-macrophage Colony Stimulating Factor and Interleukin-4. After a five day culture, DC were infected with either live or irradiated S. aureus for two hours. Irradiated S. aureus maintains its structure, but is no longer viable, whereas live S. aureus retains the ability to produce secreted proteins. Apoptosis was measured via Annexin-V/FITC and Propidium Iodide staining by flow cytometry at 24 and 48 hours post-infection. Lower levels of apoptosis were measured in DC infected with live S. aureus as compared to uninfected DC. Irradiated S. aureus induced more apoptosis in DC than live S. aureus. This may indicate a role for secreted proteins from S. aureus in the inhibition of DC apoptosis. Future studies will decipher the secreted proteins and identify the anti-apoptotic proteins involved in intracellular S. aureus survival. Together these data will evaluate S. aureus' ability to manipulate the host immune system and will provide a base of information from which to design more efficacious treatments.

076P
Combination DNA plus protein *Brucella canis* vaccine
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Brucella (B.) canis is mainly transmitted by direct or indirect contact with aborted fetuses and placenta. It's also known to be able to infect human, which likely results in providing vetrinarians and companion animal owners for infectious risk. For the development of an effective subunit vaccine against canine brucellosis, immunogenicity for chaperonin GroEL(heat shock protein of 60kDa) of *B. canis* was investigated by DNA prime-protein boost vaccine, that is known to induce humoral and cellular immune responses in mouse models. pcDNA3.1(+)groEL and GroEL protein vaccine was assessed for immunogenicity and protective efficacy in BALB/c mice(6 weeks old, n=66). They were divided into 4 groups including control group. The first group(DDD) were immunized intramuscularly with 50ug of only DNA vaccine on days 0, 14 and 21. The second group(SSS) were injected subcutaneously in the lower back midline with 30ug of purified GroEL on same period. The third group(DDS), that is DNA and protein vaccination were injected intramuscularly 50ug of DNA vaccine on days 0, 14 and subcutaneously in the lower back midline with 30ug of purified GroEL on day 21. Sera were obtained at 0, 20, 40 days after the first immunization to detect humoral and cellular immunity. The mice were challenged intraperitoneally with 3.2×10^6 CFU of *B. cains* ATCC23365 on 4 weeks after the last booster injection. After 4 weeks challenged, the number of *B. canis* per spleen (group, n=9) was tested to measure protection level against each DNA vaccine by the plate count method. Significantly highest IgG1 and IgG2a were detected in the sera of immunized mice with SSS as compared to those immunized with DDS or DDD. Interferon-γ and IL-2 were elicited higher level in the sera immunized mice with SSS and DDD than DDS groups. IL-4 was no significant differences among 4 groups. In mouse challenge experiment, SSS and DDD were reduced significantly the number of *B. canis* from spleen as compared to negative control groups. Further studies are required to construct a multivalent vaccine with selecting antigens against *B. canis*.

077P
Macrophage extracellular trap formation in response to M. haemolytica or its LKT is altered by co-incubation with bovine herpes virus-1 infected bronchiolar epithelial cells
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Bovine Respiratory Disease (BRD) is the primary cause of morbidity in the U.S. beef and dairy industries. BRD is caused by viral and bacterial agents that lead to severe pleuropneumonia in cattle, which is characterized by inflammation, intense neutrophil infiltration, consolidation and recently, extensive amounts of extracellular DNA in the lungs. One possible source of this DNA is from leukocytes that release fibrillar network referred to as extracellular traps (ETs). Previously, it was demonstrated that neutrophils and macrophages produce ETs in response to *Mannheimia haemolytica* and its leukotoxin

077P (continued)
(LKT). Conditioned media removed from bovine herpes virus (BHV)-infected bovine bronchiole epithelial (BBE) cells contained several cytokines including interferons, IL-1 and IL-6. Therefore, we examine if conditioned media from BBE cells infected with BHV affect ET formation from bovine neutrophils or macrophages. BBE cells were incubated with BHV for various times and conditioned media was removed and stored for further use. Leukocytes were co-incubated with conditioned media and *M. haemolytica* cells or its LKT for various amounts of time and ET formation was measured using PicoGreen fluorescence. Our data reveal a decrease in ET formation when conditioned media was co-incubated with macrophages and *M. haemolytica* cells or LKT in comparison to the control. However, neutrophils were unaffected by the co-incubation. Our findings suggest that BHV infection may cause a decrease in *M. haemolytica*- or LKT-induced ET formation, which could alter host defense and inflammation.

078P
Brucella abortus recombinant outer membrane proteins induce clearance immunity against virulent challenge in BALB/c mice.
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We previously described the ability of a Type-V outer membrane protein, Omp25d, to induce clearance immunity in the mouse model for *B. abortus* infection (Leonhardt, et al. 2010. 91st Annual CRWAD). In this present study, we evaluated an additional *Brucella* Type-V protein, Hia, and an outer membrane protein up-regulated in vivo, D15, in the mouse vaccine-challenge model. The two recombinant antigens were expressed and purified from *E. coli*, and 30 µgs of each were formulated with either aluminum hydroxide (Alhydrogel™) or a lipid-based adjuvant (TiterMax™) prior to subcutaneous immunization in 6-week old BALB/c mice. After a two-dose vaccine regimen, mice were challenged by interperitoneal route with 5 x 10^4 CFU wild-type *B. abortus* strain 2308, spleens excised at 7, 14, and 21 days post-exposure, and bacterial loads determined by colony count. At 7 days post-challenge, no differences in splenic bacterial loads were observed in either the Hia or D15 + Alhydrogel-vaccinated animals compared to the adjuvant-only controls. At day 14 however, while a reduction in 0.5 log was seen in the Hia- immunized mice, bacterial loads were more dramatically reduced in D15 + Alhydrogel-vaccinated animals (>3.0 logs, $p<0.001$) relative to the negative controls. By day-21, splenic colonization in Hia and D15 -vaccinated mice were reduced by 2.5 and 1.5 logs respectively relative to the Alhydrogel adjuvanted-only controls. In contrast to Alhydrogel-formulated Hia, animals immunized with the TiterMax-formulated protein possessed a 1.7 log reduction in bacterial loads early after infection (7 days), although the pathogen appeared to "rebound" at later time points, showing no differences in bacterial loads compared to the controls at days 14 and 21 post-challenge. We conclude that both Hia and D15 outer membrane proteins have potential as sub-unit vaccine candidates against *B. abortus* infection/colonization when formulated with a salt-based adjuvant. The immunological basis for differences in clearance effects seen with Hia formulated with the two different adjuvants is under investigation.

079P
Granzyme B release is triggered by activation of bovine lymphocytes
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Mastitis is an infection of the mammary gland that costs the US dairy industry 2 billion dollars yearly in losses. One of the causes, Staphylococcus aureus, has no effective vaccine in the market. We propose that an efficient cellular based vaccine against S. aureus in bovines should induce a memory response against the pathogen. An effective memory response will include effector memory cells that release specific enzymes like Granzyme B. Granzyme B is secreted by cytotoxic T cells, and can help reduce the bacterial load during infection in the mammary gland. To study the memory response against S. aureus, monocytes were collected from animals previously infected with S. aureus and animals with no previous clinical infection, and differentiated into dendritic cells (DC). The DC were loaded with live or gamma irradiated S. aureus and used to present the antigens to lymphocytes from the same animals. The supernatants from co-cultures were collected and tested for active Granzyme B to assess the response of lymphocytes to S. aureus antigens. Lymphocytes from previously infected animals produced greater amounts of Granzyme B as compared to animals with no previous infection. In animals with no previous clinical infections, irradiated S. aureus antigen induced lower levels of Granzyme B than live S. aureus. In previously infected animals, both types of antigens elicited a similar response. This data indicates that previous infection can heighten the ability of cytotoxic T cells to produce Granzyme B. Future experiments will reveal the exact S. aureus antigens responsible for the formation of memory response and effective lymphocyte response aiding in the development of a vaccine.

080P
Optimization of 6 hours intracellular cytokine flow cytometric assay using ESAT-6-CFP-10 for diagnosis of bovine tuberculosis in Egypt
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Object: Mycobacterium bovis (M. bovis.) is the causative agent of bovine tuberculosis (BTB), a disease of economic importance and human health risk. In Egypt, use of single intradermal tuberculin test (SITT) resulted in the dramatic reduction of BTB but interference of environmental mycobacteria has limited use SITT opening up the quest for more specific antigens to develop a more specific diagnostic test for BTB. Present study was conducted to develop a gamma interferon flow cytometric cytokine assay using early secreatory antigenic target-6 (ESAT-6) and culture filtrate protein-10 (CFP-10) antigens. Methods: Heparinized blood was collected from 10 SITT reactor dairy cattle and 2 control cattle reared in a TB-free herd. Blood was stimulated in the presence of anti-CD49d, anti-CD28 (1 µg/ml) and activated with ESAT-6/CFP-10 (5 ug/ml). PMA (50 ng/mL) and ionomycin (1µg/mL) were used as a positive control. Blood was incubated at 37°C in 5% CO2 for 6 hours. After 2 hours, Brefeldin A (10µg/mL) was added. Cells were stained with the following antibodies: CD3, CD4, CD45R0, CD69 and IFN-γ. Samples were run on a flow cytometer. Data were analyzed using FCS Express software (De Novo Software). Data are reported as the frequency ofCD3+ CD4+ CD45R0+ T cells that stained positive for IFN-γ and/or CD69. Results: The results showed that ESAT-6/CFP-10 was recognized by memory CD4+ T cells in TB-infected cattle that upregulated CD69. Function alanalysis showed that memory CD4+ T cells produced significant frequency of IFN-γ compared to non-infected cattle. Conclusion: The findings show that 6 hour intracellular cytokine flow cytometric assay using ESAT-6-CFP-10 can be used for diagnosis of bovine tuberculosis in Egypt.

081P
The effect of maternal colostral immune cells on neonatal health and immune development.
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Mortality and decreased weight gain resulting from infection and disease in calves is a significant problem within the dairy industry. The bovine neonate relies solely on colostrum for the acquisition of passive immunity. To date, colostrum quality is determined by concentration of antibodies. However,

081P (continued)
colostrum also contains proteins and cells which may enhance immune development in the neonate. The aim of this study was to analyze the impact of non-antibody colostral components on long-term immune development. Thirty-seven female Holstein and Jersey dairy calves were bottle-fed 4 quarts total of either fresh or frozen colostrum at birth. Treatment colostrum was flash-frozen in liquid nitrogen to destroy immune cells. Calf peripheral blood samples were taken before and after feeding colostrum as well as on day 1, 3, 7, 14, 21, 28, and subsequently once a month. Blood and colostrum were analyzed for antibody and cytokine levels by ELISA and cell profiles were determined by flow cytometry. Treatment showed no significant differences between fecal scores. However, total respiratory scores were higher for fresh-fed calves on day 24 and for frozen-fed calves on day 38. Ear and ear scores were significantly higher in frozen-fed calves on days 37-40. Cough scores were significantly higher for fresh-fed calves on days 15 and 24. Vaccination responses were evaluated using RT-PCR. Cytokines IFN-γ and IL-2 increased within 1 month post-vaccination in fresh-fed calves but not in frozen-fed calves. Feeding of fresh colostrum may impact early immune development as well as response to vaccinations. Further findings of this study will provide novel information on the impact of colostral immune cells on neonatal health.
Keywords: colostrum, passive transfer, immunity, dairy calves

082P
Association between interferon gamma production and natural resistance in *Mycobacterium bovis* naturally infected cattle.
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In this study we evaluated the natural resistance to *Mycobacterium bovis* in cattle naturally infected with tuberculosis and its association with interferon gamma production (IFN-γ). Eleven naturally infected cattle from a high prevalent herd were used. The infection from these cattle was confirmed by single intradermal comparative cervical test and PCR from nasal swabs. Peripheral blood monocytes were isolated for all the animals and were used in microbicide test to characterize the cattle phenotypically resistant or susceptible to *Mycobacterium bovis*. Furthermore, interferon gamma test were performed in order to know their IFN-γ levels. All cattle tested were susceptible to *Mycobacterium bovis*, showing an apparent difference in susceptibility between individuals. On the other hand, cattle also showed different levels of IFN-γ; although, there were no statistical difference between them, which perhaps might suggest that there is not a relationship between the production of IFN-γ and susceptibility to *Mycobacterium bovis*.
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083P
A novel diagnostic tool for horses with pituitary pars intermedia dysfunction (PPID)
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Over the past decade the aged horse population has expanded significantly with 20-30% of the equine population comprised of geriatric horses (≥ 15 years). As a result there is a mounting demand for veterinary care and management of these aged horses. Unfortunately, one of the most common equine endocrinopathies, affecting 30% of older horses, is Equine Cushing's disease (ECD), also known as pituitary pars intermedia dysfunction (PPID). Diagnosis of PPID is largely recognized as being complicated and lacking of a gold standard diagnostic method that will allow for high sensitivity despite seasonal changes. Hence, there is confusion among equine clinicians regarding the best way to diagnose PPID. Thus, we hypothesized that a novel diagnostic test will be superior to commonly used diagnostics for PPID. PPID horses were identified by signs of hirsutism and significantly ($p<0.05$) elevated resting plasma alpha-melanocyte-stimulating (a-MSH) levels (>30 pmol/L = PPID) which is a gold standard measure. Heparinized plasma was harvested from blood samples collected from normal (n=15) and PPID (n=10) horses (≥20 yrs) and frozen until subsequent assessment of basal prolactin, adrenocorticotropin (ACTH) and (a-MSH) hormones using radioimmunoassay (RIA). Comparing resting plasma prolactin levels in normal (n=15) and PPID horses (n=10), there was a significant difference ($p<0.05$) (Fig 1A). Spearman correlation revealed a significant correlation relationship between prolactin and a-MSH ($p=0.003$, $R=0.669$), but not prolactin and ACTH ($p=0.410$, $R=0.225$), nor , a-MSH and ACTH ($p=.465$, $R=.2$). Here, we provide steps towards identifying a novel diagnostic tool for equine clinicians and diagnostic labs to diagnose PPID.

084P
Comparison of nutritional compounds (pterostilbene, resveratrol, curcuminoids, quercetin, and hydroxypterostilbene) to NSAIDs on equine cytokine production in vitro
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Advanced age in horses (>20 years) is associated with increased production of pro-inflammatory cytokines, termed "inflamm-aging". Nutritional intervention to counteract this response in old horses remains to be studied. Non-steroidal anti-inflammatory drugs (NSAIDs) such as flunixin meglumine and phenylbutazone are commonly used to treat inflammation in horses. However, long term use of NSAIDs can pose health problems in the horse. Thus, alternative use of nutritional compounds including pterostilbene, resveratrol, curcuminoids, quercetin, and hydroxypterostilbene are of interest. We hypothesized that the nutritional compounds would significantly reduce inflammatory cytokine production similar to NSAIDs. The aged horse was used as a characterized model of systemic inflammation to determine the effects of these compounds on in vitro cytokine production. Heparinized blood was collected aseptically via venipuncture from aged horses. PBMCs were isolated and cultured in vitro with each of the compounds at varying doses (10-4, 10-5M) and then stimulated with PMA-ionomycin. IFN-gamma and TNF-alpha production were measured by flow cytometry. Cytokine gene expression was also analyzed by RT-PCR. Results indicated that there was an overall significant difference among treatment groups for production ($P<0.05$). Resveratrol, quercetin, and flunixin meglumine (10-5M) reduced cytokine production compared to the other compounds. This study provides a preliminary step towards identifying nutritional compounds that may have the ability to alter inflammation in the horse.

085P
Immunogenic ability of a recombinant QseC, a bacterial adrenergic receptor, to induce innate and adaptive immune responses in avian macrophages
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Quorum sensing is a cell-to-cell signaling mechanism used by various species of bacteria to co-ordinate the gene expression in response to chemical hormone-like molecules called autoinducers. The QseC sensor kinase serves as a bacterial adrenergic receptor to these autoinducers and regulates virulence in multiple Gram- negative bacteria. QseC is an important component of a two-component regulatory system in bacteria, wherein QseC represents the histidine kinase (HK) and controls the activity of its response regulator QseB. QseC acts as a bifunctional sensor kinase/phosphatase that controls the phosphorylation state of QseB, and thereby maintains optimal gene expression. More recently QseC has been reported to control the central metabolic

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085P (continued)
circuit of pathogenic *E. coli*. Like most of the other HK's, QseC is a membrane-bound sensory kinase. Given the role that QseC plays in the bacterial virulence, targeting its activity via generation of either innate or adaptive immunity could be a logical approach to control a wide range of QseC-bearing pathogens. However, the ability of QseC protein to stimulate immune responses is still unknown. With this research in mind, present study evaluated the immunogenic ability of a recombinant QseC protein to stimulate innate or adaptive immune responses in avian macrophages *in vitro*. Avian macrophages were treated with purified native form of the QseC protein expressed in *E. coli* and the innate and adaptive immune responses were evaluated. Our results demonstrate that QseC is immunogenic and could possibly be considered as a sub-unit vaccine candidate, however further validation using *in vivo* animal models is required.

086P
Magnetic resonance microscopic imaging of hearts reveals structural and functional defects in autoimmune myocarditic mice.
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Myocarditis is an inflammation of the myocardium, but only ~10% of those affected show clinical manifestations of the disease. Nonetheless, myocarditis is still regarded as an important cause of heart failure in children and adolescents, particularly athletes, indicating that a low degree of inflammation could be present in the hearts of apparently healthy individuals. To study the immune events of myocardial injuries, various mouse models of myocarditis have been widely used. Our studies involve experimental autoimmune myocarditis (EAM) induced with cardiac myosin heavy chain (Myhc)-α 334-352 in A/J mice and the affected animals develop lymphocytic myocarditis but with no apparent clinical signs. We report here application of magnetic resonance microscopy (MRM) as a non-invasive modality to determine the cardiac structural and functional changes in animals immunized with Myhc-α 334-352. EAM and healthy mice were imaged using a 9.4 T (400 MHZ) 89 mm vertical core bore scanner equipped with a 4 cm millipede RF imaging probe and 100 G/cm triple axis gradients. Cardiac images were acquired from anesthetized animals using a gradient-echo-based cine pulse sequence and the animals were monitored by electrocardiography, respiration and pulse oximetry. The analysis revealed that the thickness of ventricular wall was increased in EAM mice by 1.5 fold including the heart rate with a corresponding decrease in the interior diameter of ventricles when compared with healthy mice. Histologically, hearts from EAM but not healthy mice showed multifocal lymphocyte infiltrates suggesting that morphological and functional changes in the inflamed hearts can be monitored by MRM non-invasively in live animals. In conclusion, MRM offers an advantage of assessing the progression and regression of myocardial injuries in diseases caused by infectious agents including response to therapeutic interventions.

RESPIRATORY DISEASES POSTERS

087P
The use of a new porcine epithelial cell model for the study of PRRSV-PCV co-infection reveals a PCV genotype dependent effect
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Porcine circovirus (PCV) type 2 (PCV2) is a major pathogen in the swine industry and has been described as the causative agent of a list of conditions under the designation of porcine circovirus-associated diseases (PCVAD). Attempts to replicate PCVAD initially failed, as it was discovered that an immune trigger could facilitate the reproduction of clinical signs, either by co-infecting with other swine pathogens or using immunomostimulants. As such, contributing pathogens are commonly isolated in PCVAD field cases. Porcine reproductive and respiratory syndrome virus (PRRSV) is the most frequently isolated agent in post-weaning multisystemic disease and circovirus-associated necrotic pneumonia.
To study the importance of this trigger, we developed an in vitro model which allows the replication of both PRRSV and PCV. This model was used to compare the effect of PRRSV on PCV replication and their impact on apoptosis and innate immune response. A neonate porcine tracheal cell line (NPTr) was genetically modified to stably express CD163 (NPTr-CD163), an essential PRRSV surface receptor.
NPTr-CD163 cells were able to replicate all PCV genotypes (PCV1, PCV1/2a, PCV2a and PCV2b) and PRRSV type II IAF-Klop strain. A significant effect of PRRSV on PCV replication is found to be genotype dependent, as PCV1 replication is down regulated with the presence of PRRSV and PCV2b replication is up regulated compared to PCV infection alone. Inversely, PCV1 increases PRRSV replication, whereas PCV1/2a, PCV2a and PCV2b significantly lower PRRSV replication. Cytokine mRNA expression analyses showed that TNF-α and IFNβ were significantly increased in the PCV1-PRRSV co-infection model compared to single infections, whereas they remained low for the other genotypes.
These results suggest an interaction between that is PCV genotype dependant. PRRSV increases significantly the replication of PCV2b. This may explain the significant increase of PCVAD clinical cases in North America that appeared in 2005 and which was associated with the emergence of PCV2b. The level of virus replication and the porcine tracheal origin makes this cell line an interesting in vitro model for the study of PRRSV-PCV co-infection.

088P
Development of an immortalized canine respiratory epithelial cell line for canine influenza virus infection
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Purpose: Canine influenza is an emerging disease mainly caused by H3N8 and H3N2 influenza A viruses originated from equine and avian species, respectively. It causes an acute respiratory distress in dogs. Madin-Darby Canine Kidney (MDCK) cell line is usually used to isolate canine influenza virus (CIV) and analyze pathological studies in vitro. However, MDCK cells would not represent respiratory cell responses to the CIV infection because they are kidney epithelial cells. Here we developed a canine respiratory epithelial cell line to study proper cellular responses to the CIV infection.
Methods: Primary epithelial cells were removed from canine bronchia with protease XIV and cultured by serum-free bronchial epithelial growth medium (BEGM). The primary cells were transfected with the pSV3-neo plasmid carrying a Simian Virus 40 large T-ag sequence. The transfected cells were selected at 7 days post transfection with G 418 and growing cells were cloned by limiting dilution.
Results: One clone, namely KU-CBE cells, was finally selected. KU-CBE cells express cytokeratin 18, an epithelial cell marker, in the cytoplasm and SV40 large T-ag, an immortalization-inducing viral onco-protein, in the nucleus. KU-CBE cells were permissive to H3N2 canine influenza virus. The growth curve patterns of CIV were compared with KU-CBE and MDCK cell lines.
Conclusions: In this study, we developed for the first time an immortalized canine bronchial epithelial cell line. We expect the newly established KU-CBE cells would be broadly used to study CIV infections and other canine respiratory diseases.

089P
Protection effect against PRRSV infection and boosting effect on PRRSV vaccine of immunostimulator(Barodon®) in pigs
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089P (continued)
Barodon®(Barodon-S.F, Korea), has been introduced for its effectiveness as a nonspecific immunostimulator in pigs. PRRSV is the most significant infectious disease currently affecting the swine industry worldwide. The purpose of this study is to evaluate protection effect against PRRSV infection in pigs. 40 weaning pigs were divided into non-vaccine groups and vaccine groups largely. Groups excepted control were fed with Barodon® according to concetnration of Barodon®. Non-vaccine groups were divide into NV-A(control), NV-B(0.025%), NV-C(0.05%) and NV-D(0.1%). Vaccine groups were divide into V-A(control), V-B(0.025%), V-C(0.05%) and V-D(0.1%). Total experimental period was 8weeks. Vaccine groups were inoculated PRRSV vaccine(Behringer Ingelheim, Germany)at 4weeks. The pathogenic US PRRSV($10^{5.5}TCID_{50}$/ml) was inoculated via nasal cavity(2ml) and trachea(2ml) at 7weeks. Clinical sign and average daily gain(ADG) were checked for the experimental period. Blood samples were collected at 4, 7, 8, 9, 10 and 11 weeks for ELISA. Nasal swap was conducted at 7, 8, 9, 10 and 11 weeks to detect excretion of PRRSV. After necropsy, lungs were observed to check gross lung lesion. Respiratory organ samples (tonsil, hilar lymph node and lung) were collected for detection of PRRSV and quantitative analysis by real-time PCR. H&E stain was used in the lungs for measuring microscopic interstitial lung lesion.
In ADG, the results of NV-C and NV-D were higher than those of NV-A, significantly. Antibody titers of NV-C and NV-D increase rapidly after PRRSV inoculation. Antibody responses of V-C and V-D fed barodon were boosted at 4-7weeks after PRRSV vaccination, significantly. In quantitative analysis of PRRSV, the results of NV-C and NV-D were lower than those of NV-A in hilar lymph node. In other inspection items, groups fed Barodon® showed results improved comparing with control, although there were no significant difference in experimental groups.
In this experiment, feeding supplemented with Barodon® had an effect to improve ADG and antibody response and reduce respiratory clinical signs, interstitial lung lesion, concentration of PRRSV in respiratory organs.

090P
Barn dust exposure impairs swine alveolar macrophage function: implications for swine respiratory health
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Respiratory diseases are responsible for a significant amount of animal morbidity and mortality in the swine industry, including the highest percentage of all nursery deaths and the majority of grower/finisher deaths. Innate immunity, including the maintenance of lung macrophage health and function, is an important defense mechanism against respiratory pathogens and their associated losses. Chronic exposure of swine industry workers to airborne barn dust results in significant predisposition to airway diseases including rhinitis, bronchitis and obstructive pulmonary disease. To date, few studies have been performed to determine if barn dust exposure negatively affects the swine immune system, and none have directly tested whether dust affects macrophage phenotype or function. We sought to determine how barn dust affects swine macrophages by defining the immune alteration(s) in cytokine production and cell surface marker expression, as well as their functional and antibacterial capacity. An organic dust extract (ODE) was gifted from the University of Nebraska Medical Center where it was prepared by suspending dust collected from a swine confinement facility in solution, clarified by centrifugation and filter sterilized. This is the first study to show that exposure of pig alveolar macrophages to swine barn ODE induced the secretion of both pro- and anti-inflammatory cytokines from pig lung macrophages, including IL-1β, TNF-α, and IL-10, suggesting a complex activation profile. Exposure to ODE enhanced the expression of several cell surface markers of activation, including a receptor for porcine reproductive and respiratory syndrome virus (PRRSv). ODE exposure diminished phagocytic uptake of particles, a critical function of alveolar macrophages. The ability of alveolar macrophages to kill a respiratory isolate of Salmonella enterica serovar Choleraesuis was also decreased after ODE exposure. Taken together, these results indicate that dust exposure negatively affects macrophage function and alters their immune phenotype, potentially enhancing host susceptibility to a variety of respiratory infections.

091P
In vitro biofilm formation by *Mannheimia haemolytica*
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Mannheimia haemolytica is an opportunistic bacterial pathogen, found in the upper respiratory tract in cattle, that causes economic losses for the dairy and beef industries. Very little is known about how M. haemolytica adheres to respiratory epithelial cells, which is a crucial event in pathogenesis of bovine respiratory disease. We hypothesize that once the bacterial cells adhere to the respiratory epithelium, they create biofilms that protect them and allow them to persist in vivo. In this investigation, we focused on several culture conditions to determine their effects on M. haemolytica biofilm formation in vitro. We incubated the bacteria in various growth media and found that RPMI tissue culture medium resulted in the greatest amount of biofilm formation. We next asked whether the bacterial cells formed more biofilm with or without 5% CO_2 and observed that the bacterial cells formed greater amounts of biofilm under CO_2 incubation at 37°C. We also observed a dose-dependent inhibition of biofilm formation with increasing concentrations of fetal bovine serum. Ongoing experiments are comparing biofilm formation on plastic surfaces coated with various extracellular matrix proteins (e.g. collagen, fibronectin, laminin). These findings will help define a model system for studying events that influence adhesion and colonization of M. haemolytica to bovine respiratory epithelial cells.

092P
The kinetics of white blood cell counts during vaccination against Bovine Respiratory Disease pathogens and their correlations with lung lesions, diagnosis and average daily gain.
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Bovine Respiratory Disease (BRD) is the most common disease within US feedlots. Infection can result in morbidity, mortality and reduced average daily gain. The discovery of cheap and reliable methods of prediction and/or protection would be highly advantageous to both breeders and farmers. Cattle (2182) were vaccinated against common viral and bacterial pathogens of BRD. Two blood samples were collected; during booster vaccination and 21d later, enabling 3 phenotypes for each trait (Pre, Post and Delta [Post - Pre]). From the blood samples innate and adaptive responses (counts of: white blood cells (WBC), neutrophils (NE), lymphocytes (LY), monocytes (MO), eosinophils (EO) and basophils (BA)) were measured. In addition, feedlot average daily gain (ADG), health records (HR) and lung scores (LS; collected at harvest) were also measured. Traits ADG, HR and LS have all been significantly correlated with infection to BRD. In this investigation we aimed to find correlations between the immune response and ADG, HR and LS; finding an easily measurable trait which would be a good predictor of the efficacy of vaccination. The results showed a positive Delta for the innate immune response (EO, BA, NE), while the adaptive immune response had a negative Delta (LY). Overall, we discovered that the immune responses had moderately high heritabilities (lowest: Delta MO, 0.21; highest Pre LY: 0.5), with LY having the highest h^2 throughout the study ($h^2 \geq 0.41$). All significant genetic correlations were calculated using bivariate REML models. While LS did not significantly correlate with any of the immune phenotypes, both ADG (LY, -0.24) and HR (Pre EO, -0.67; Delta WBC, -0.5 and Delta LY, -0.67) did. Interestingly all the significant genetic correlations were negative, suggesting

RESPIRATORY DISEASES POSTERS

092P (continued)
successful immunization of animals appears to be a function of a high level of LY pre-booster and a low negative (or positive) Delta, of WBC and LY, 21 days after the booster vaccination is administered. The increase in EO and BA may potentially link their role in decreasing LY. These results may enable farmers to quarantine, re-vaccinate and breed animals to lower the incidence of BRD post-vaccination.

VECTOR-BORNE AND PARASITIC DISEASES POSTERS

093P
Avian hemoparasites in Illinois and their effects on health
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Purpose: The objectives of this study are (1) to assess basic health parameters (white blood cell counts, packed cell volume, glucose, hemoglobin and plasma proteins) between infected and non-infected upland game and waterfowl and (2) to determine the type, prevalence, density and intensity of hematozoa under natural conditions.
Methods: Blood samples were obtained from game birds, blood smears were created and the remaining blood was submitted to the University of Illinois' College of Veterinary Medicine for blood chemistry and complete blood cell counts. Smears were analyzed for parasites and results from the veterinary school were analyzed with respect to those results.
Results: Only low intensity infections were detected in 2012, infected birds showed no statistically significant differences in any health parameters compared to non-infected individuals.
Conclusions:Low intensity parasite infections do not induce clinical indications of immune stress. Continuation of surveying is recommended to further understand to health impacts of high intensity infections.

094P
Detection of *Bartonella* species from cattle ticks in South Korea
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Purpose: In South Korea, *Bartonella* species have been previously detected in ticks, rodents, and small mammals, but there was no report of bartonellosis in cattle. We report the presence of *Bartonella* species in cattle ticks and provide the genetic relationship between these species and *Bartonella* isolates registered in GenBank.
Methods: During 2010 - 2011, ticks were removed from cattle from five different provinces of Korea (Gyeongbuk, Chungbuk, Jeonbuk, Jeonnam, and Jeju) in South Korea by using tick twister (Tick Twister®, France). Microscopic observation of all 877 ticks collected allowed for species-level classification and pooling into 556 samples. Genomic DNA was extracted from the ticks by using the QIAamp DNA extraction kit, and a PCR assay was performed using the BTNi-F and BTNi-R primer set, which targets the 16S RNA gene for *Bartonella* species. After cloning and enzymatic digestion, phylogenetic analysis was accomplished using the MEGA 3.1 software.
Results: Among 877 ticks, 874 ticks were identified as *H. longicornis* (99.66%), and three (0.34%) were identified as *Ixodes* species. They were mainly collected from the southern part of South Korea, that is, from Jeju Island (44.5%) and the Jeonnam province (28.3%). A 16S RNA PCR assay for *Bartonella* species yielded a specific 356-bp amplicon for nine of the 556 pooled DNA samples. A phylogenetic tree based on the partial sequencing of 16S RNA was constructed using neighbour-joining analysis with MEGA 3.1 and showed two clusters of *Bartonella* strains.
Conclusions: Cluster A included four isolates that were very similar to those previously registered on GenBank obtained from various sources, the cluster B isolates were highly identical to *B. henselae* from a human patient. Therefore, we can infer that *H. longicornis*, harbouring various *Bartonella* species, might circulate among human beings (farm owner and workers); cattle; rodents; nest; and composts near the farm. Because ticks are one of the main vectors that transfer pathogens affecting human beings and domestic cattle, further studies are necessary for investigating epidemiological relatedness between ticks and these pathogens and surveying bartonellosis in cattle.

095P
Effect of skin lesion on Haematological picture of some dogs in Ibadan.
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The study reported the effect of skin diseases on haematological parameters in dogs presented in some veterinary clinics in Ibadan. 44 out of the 80 dogs (different breeds) presented during the period were positive for mange infection (32 sarcophilic, 10 demodecdic and 2 mangelic) 2 mls of blood was collected from the cephalic vein of dogs that had skin lesion with apparently healthy dogs without skin lesions. The result obtained shows that Hb, PCV and RBC count of infected dogs (54.5%) fall within the range of 7.8-11.7g/dl (10.9g/dl),26-34% (30.6%) and 3.42-4.01x$10^{6\ ul}$ (3.4x10^{6}_{ul}) respectively which is slightly lower than 11.8-14.7g/dl (11.9g/dl), 36-45% (35.9%) and 5.25-6.90x10^{6}_{ul} recorded for the control. This indicates mild to moderate anaemia in these dogs. The study shows that there is a significant different $p \leq 0.05$ in the PCV, value of dogs with skin lesion when compared with normal dogs. The skin lesions in dogs affect PCV value in dogs.

VIRAL PATHOGENESIS POSTERS

096P

Clathrin-mediated endocytosis is required for porcine epidemic diarrhea virus entry into Vero cells

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In this study, we evaluated the entry mechanism of PEDV into Vero cells. Our data reveal that PEDV entry into Vero cells follows clathrin-mediated endocytosis pathway independence of caveolae-coated pit assembly. It was colocalized with the clathrin-mediated endocytic marker, but not with the caveolae-mediated endocytic marker. Cells treated with lysosomotropic agents were resistant to PEDV, suggesting that a low-pH-dependent step is required for the fusion of its envelope with the plasma membrane. Inhibitor for the endosomal serine proteases greatly reduced PEDV entry while the cysteine, aspartic, and metaloproteases inhibitors had little effect on PEDV infection. These suggest that there was a serine proteolytic step in clathrin-mediated endocytic entry. These findings demonstrate that PEDV enters Vero cells through the clathrin-mediated endocytosis and requires low pH. The entry mechanism of PEDV is similar to that described on severe acute respiratory syndrome coronavirus and mouse hepatitis virus rather than to other *Alphacoronaviruses* with some differences. As the conclusion, PEDV is highly syncytial, but only if cleaved first.

097P

Effectiveness of small interfering RNA (siRNA) to inhibit feline coronavirus replication

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Purpose: Feline infectious peritonitis (FIP) continues to be a significant cause of mortality in cats. Feline Coronavirus (FCoV), the agent of FIP, primarily targets intestinal epithelial cells, but in certain cats virus mutation may occur that allows the virus to replicate efficiently in monocytes and macrophages, resulting in FIP development. One afflicted, there is no treatment to prevent progression to death. In this study, we evaluate the ability of siRNA to inhibit the in vitro viral replication and gene expression of FCoV.
Methods: Four synthetic siRNAs targeting four different regions of the FCoV genome were tested for their antiviral effects against two different strains of FCoV; FIPV WSU 79-1146 and FECV WSU 79-1683. Efficacy was determined by real time RT-PCR of intracellular viral genomic RNA and flow cytometry for viral protein expression.
Results: The four siRNAs exhibited a variable inhibitory effect on the two viral strains. siRNAL that target the leader gene resulted in 95% and 99.5% reduction in FIPV WSU 79-1146 protein and RNA expression respectively as well as 83% and 96% reduction in FECV WSU 79-1683 protein and RNA expression respectively. siRNAM that target the membrane gene resulted in more than 40 % reduction in protein expression of the two strains.
Conclusions: These preliminary findings shows that FCoV translation and replication can be specifically inhibited using siRNA targeting coding and noncoding region of viral genome, suggesting a potential therapeutic application of RNAi in treating FIP.

098P

Construction and characterization of infectious clone of an interferon-inducing PRRSV strain

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Porcine reproductive and respiratory syndrome virus (PRRSV) is known to interfere with interferon signaling. PRRSV A2MC2 was discovered to induce type I interferons in cultured cells. The objective of this study was to construct an infectious clone using reverse genetics technology. RNA was extracted from A2MC2 virions and used in reverse transcription to synthesize cDNA. The full length cDNA of A2MC2 was cloned into a plasmid vector. Ribozyme sequences were inserted upstream and downstream of the A2MC2 cDNA for a DNA-launched infectious clone. DNA sequencing confirmed the sequence of A2MC2 in the plasmid. Transfection of MARC-145 cells led to recovery of virus from the A2MC2 plasmid. The rescued virus appeared similar to its parent strain in terms of induction of type I interferons and of growth properties in cultured cells. To test its replication and virulence in piglets, the rescued virus and parent A2MC2 were used to infect pigs. Viremia, antibody level and lung pathology are being analyzed. Construction of this infectious clone will be useful for molecular characterization of A2MC2 and its mechanism in induction of interferons.

099P

The nonstructural protein 1 of murine arterivirus lactate dehydrogenase elevating virus is a viral type I interferon antagonist

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Lactate dehydrogenase elevating virus (LDV) is a murine arterivirus and along with PRRSV belongs to the family Arteriviridae. LDV persists in infected mice without notable pathological changes and clinical signs. Since the porcine arterivirus PRRSV is able to suppress the type I interferon (IFN) response during infection and the type I IFN plays a pivotal role for establishment of persistent infection, we investigated whether LDV contained an ability to down-regulate IFN response as with PRRSV. The Nsp1 protein of LDV was cleaved into two subunits, Nsp1α and Nsp1β, and both subunits were found to localize in the cytoplasmic and nuclear compartments of the cell. The inhibition of IFN-β induction was observed when Nsp1α or Nsp1β was expressed in cells, and the inhibition was mediated through the interferon regulatory factor 3(IRF3) and NF-κB pathways. Phosphorylation of IRF3 remained unchanged during expression of Nsp1α and Nsp1β, indicating that LDV Nsp1 did not induce IRF3 degradation nor inhibit IRF3 phosphorylation. CREB (cyclic AMP responsive element binding)-binding protein (CBP) is the key molecule recruited by IRF3 to form enhanceosome to instigate IFN production, and CBP was found to be degraded by LDV Nsp1. When two subunits of Nsp1 were examined, the Nsp1β subunit did not cause CBP degradation and only the Nsp1α subunit contributed to CBP degradation. Our data show that Nsp1 of LDV participates in the IFN modulation and suggests that the IFN suppression may be a common strategy for arterviruses to antagonize the host IFN response during infection.

100P

Biological properties of low pathogenic influenza A viruses isolated from wild birds in the Black Sea region of Ukraine

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Constant monitoring of the epizootic situation in wild birds for influenza A is one of the main components of the system of control, early warning and forecasting of epizootic situation of influenza, as many species of wild birds are the natural reservoir and vector of the virus in nature. In addition, these studies give possibility to get valuable information about the features of the circulation and ecology of influenza A viruses in different regions of the world and the possible introduction of new viruses into new geographic regions, as well as the emergence of new viruses with new properties, which can pose a threat to human health. An important step in this research is an in-depth study of the biological properties of influenza viruses isolated from wild birds, as it gives the opportunity to study in detail the biology of the pathogen, as well as to detect early changes that can be dangerous in the future. In the period from

VIRAL PATHOGENESIS POSTERS

100P (continued)
2009 to 2011 in the Black Sea region of Ukraine we conducted a large-scale monitoring of the circulation of influenza A viruses in wild bird populations of different ecological groups *Pelecaniformes, Ciconiiformes, Anseriformes, Galliformes, Gruiformes, Charadriiformes, Coraciiformes, Passeriformes*. Infection of wild birds with many kinds of influenza viruses A varied indifferent seasons and different years from 0.42 to 1.83%. As a result of virological studies there was isolated low pathogenic influenza A viruses of different subtypes. Thus the study of the biological properties of low-pathogenic avian influenza A subtypes H4, H5 and H13 found that 40-50 days old chickens infected with intranasal, these viruses do not cause their disease. When virological investigations of the internal organs of the influenza virus also has not been identified. When serological studies it was established that in response to intranasal infection in 20-40% of the birds in 7-14 days after exposure to work enough specific antibodies.

101P
Phylogenetic studies of Ukrainian NDV isolates
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Purpose: Conducting phylogenetic studies of NDV strains isolated from wild and domestic birds in Ukraine between 2002 and 2011.
Methods: The 374 bp region of F gene nucleotide sequences were determined by using a RT-PCR/sequencing approach. The phylogenetic analysis was performed with using the Neighbor-Joining Method.
Results: Phylogenetic analysis indicated that the 4th Ukrainian isolates (NDV/Chicken/Lyubotin/2003; NDV/Chicken/LipovaDolina/2002, NDV/Chicken/Ivano-Frankivsk/58/2007 and DV/Chicken/Kharkiv/66/2007) was clustered together and belonged to the subgenotype VIId. Genotype VII is the genotype most frequently associated with outbreaks of ND in the Middle East and Asia.
Two strains Pigeon/Ukromne/3-26-11 and Pigeon/Dnipropetrovsk/1-18-11, which were isolated from pigeons, were placed in genotype VI. They have demonstrated 98 % homology in sequences from Russian isolates. Genotype VI contains only virulent viruses and are the predominant genotypes circulating worldwide.
The last strain was isolated from white-fronted Goose and clustered together with NDV from genotype XIV that includes prevalent in Central and West African countries in recent years NDV strains.
All of the sequences of the fusion protein cleavage site have ^{112}R/K-R-Q-K-R-F^{117} motifs, which is typical for highly virulent NDV.
Conclusions: This study has shown that velogenic NDV of VI, VII and XIV genotypes circulate in Ukraine. As known, Ukraine has unique geographical location in the center of Europe and through its great territory passes transcontinental migration routes of wild birds. The case of genotype XIV has significant epidemiological consequences for the following control of NDV in Europe. And it is necessary to continue the epidemiological monitoring since the spread of NDV among migratory and synanthropic birds poses a serious threat to commercial poultry industry.

102P
Mutation in noncytopathic BVDV persistently infected animals to generate cytopathic pair is a rare event where one animal developed a cytopathic virus that hit all the animals within one herd.
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/>Bovine viral diarrhea virus (BVDV) is positive single stranded RNA virus belonging to Flaviviridae family. BVDV has a wide host range including all ruminants. BVDV is classified into two genotype, type 1 and type 2, based on 5' UTR conserved region and two biotypes based on effect of virus on cell culture. Cytopathic BVDV induces cytoplasmic effect in cell culture in contrast; noncytopathic BVDV establishes inapparent persistent infection in cell culture. Noncytopathic BVDV cause persistent infection in calves when it became infected between 40-120 days of gestation. Cytopathic stains of BVDV arise from noncytopathic strains due to mutations of the noncytopathic strain. The mixed infection of cytopathic and noncytopathic stains results in the fatal mucosal disease. In this study we investigated the molecular changes and mechanism that lead to the development of mucosal disease within a herd of persistently infected (PI) animals. The PI noncytopathic viral sequences were identified as 2a strain with two variants (both variants were highly conserved in 5'UTR and only slightly less conserved in the E2 sequence) that infected the original bred herd. After isolation by plaque purification followed by limiting dilution we sequenced cytopathic viruses from 12 animals (6 from each non-cytopathic group) we found an insertion in the NS2-3 region which matches DnaJ cellular protein. Looking at the sequences indicates that one animal developed a cytopathic virus that went through and killed all the animals. The phylogenic analysis of cytopathic sequences revealed four groups with similarity in the NS2-3 region ranges from 92% to 98%. This is the first report that indicates the mutation in PI animals is a rare event and the source of the cytopathic virus within one herd was one virus.

103P
Non adherent cd14 negative bovine monocyte derived dendritic lose their capability to produce infectious bovine viral diarrhea virus (bvdv) during its development
M.K.S. Rajput[1], L.J. Braun[1], M.F. Darweesh[1], J.F. Ridpath[2], W. Mwangi[3], A. Young[1], C.C.L. Chase[1]; [1]Department of Veterinary and Biomedical Sciences, South Dakota State University, Brookings, SD, USA, [2]Ruminant Diseases and Immunology Research Unit, National Animal Disease Center, Agricultural Research Service, United States Department of Agriculture, Ames, IA, USA, [3]Department of Veterinary Pathobiology, Texas A&M University, College Station, TX, USA.

The dendritic cells (DCs) monitor, process and present antigen to T-cells. DC activates and regulates both innate and adaptive immune responses by their pro- and anti-inflammatory cytokines and antigen presentation to T cell. Viruses that infect DC can have a devastating impact on the immune system. In this study, the comparative ability of BVDV to replicate in bovine monocyte and MoDCs (monocyte derived dendritic cells) was measured. Monocytes were isolated and differentiated into MoDCs using bovine recombinant IL-4 and GMCSF and confirmed morphologically and phenotypically to be MoDC. Morphologically the MoDCs had long dendrites with 5-7 times larger size then monocytes. The cell surface phenotype of MoDC was CD14$^-$, CD21$^-$, MHCI$^+$, MHCII$^+$, CD86$^+$, DEC205$^+$.
For comparison, 4 strains of BVDV were used including the most virulent (1373), least virulent (28508), and a virus pair, cytopathic TGAC and noncytopathic TGAN recovered from an animal that died of mucosal disease. MoDCs remained viable 72 hrs post infection against all viruses. No infectious virus production occurred in the MoDCs while monocyte produced infectious particles. The ability of monocyte to produce infectious particle decreased with maturation. Interestingly in MoDC, viral RNA increased through 144 hr after infection. . The kinetics of viral RNA production along with the amount of viral RNA was significantly different between different viral stains. The study revealed that BVDV replicates in MoDCs but does not produce infectious particles. The ability to produce infectious virus by MoDCs was reduced with its development. Accumulation of viral RNA may have significant effect on immune response mounted by MoDCs. Future studies will be done to evaluate the effect of viral RNA accumulation in MoDCs on immune response mounted by MoDC.

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104P

Genetic variability of bovine diarrhea pestiviruses, detected in semen and veterinary drugs

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Purpose: The aim of this work was genotyping of Bovine diarrhea virus, allocated in 2 cell lines, 6 biopreparations and 4 semen samples from cattle.
Methods: Amplification of samples of cDNA from RNA of BVDV was done by Amplisans commertial kit and 5'-UTR specific priomers, published by prof. Vilcek (SK).
Results: Sequencingdata of BVDV 288 bp 5'-UTR region was shown four viral variants in analyzed group. Results demonstrated blood sera and cell lines FLK andPK15 detected viruses to first group (pairwise distances - 0,030-0,046 inside group, 0,130-0,373 - among groups), second group contained virus, allocated in vaccines (CSF, PRRS, PPV), sera for cell cultures complex globulins and cell cultures (distance in clad -0,095, intraclad distance - 0,130-0,373), third and fourth groups contained bovine semen isolates (distances - 0,200 and 0,373, respectively). Sequenced cDNA samples of BVDV, detected in cell lines of first group, were classified as 1b genotype. They were related to strains Manas and 390, respectively. Contaminants form Pestivirus origin, detected in veterinary drugs (vaccines, sera and globulin) were genotyped as 1a BVDV. They were most close situated under divergence level to strains NADL and Letuyi. Viruses of Bovine diarrhea detected in semen (Poltava та Cherv Veleten) belonged to subtypes 2a and 2b, respectively. Their topographic situation on the constructed dendrogramm demonstrated high level of homology with strains TR-2006 (Canada) and Kosice (Central Europe), respectively. Data of sequencing was published in GenBank (№№ FJ223608-FJ223614) after phylogenetic study.
Conclusions: 1a, 1b, 2a and 2b BVDVs were detected in various biological matrixes under PCR detection and sequencing

105P

Parvovirus detection in feline feces via pcr

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It has been reported that a high percentage of cats in two rescue shelters in England were shown to shed canine parvovirus (CPV-2). CPV-2 was found in feces of 32.5% of cats in an all cat shelter and in 33.9% of cats in a shelter that housed both dogs and cats. Their results showing clinically normal cats shed CPV-2 for many weeks suggest cats may be an important reservoir for maintenance of CPV-2 infection in both the cat and the dog population. However, these results were in contrast to various studies we have done during the past 15 years. In our previous studies, kittens experimentally infected with CPV-2 showed viral replication and antibody production occurred, but the virus was cleared within approximately 2 weeks. No clinical signs were seen in experimentally infected cats.
To determine if cats in a feline only rescue were shedding either CPV-2 or Feline Panleukopenia virus (FPV), we collected fecal swabs from 80 cats as well as 19 environmental samples. Our results showed that there were cats shedding parvovirus. Parvovirus shedding as demonstrated by PCR occurred only in cats that had been vaccinated with modified live parvovirus within several weeks to months previously. In contrast, cats that had not been vaccinated were not shedding parvovirus and none of the environmental samples collected from the shelter were found positive. However, finding parvovirus shed from the vaccinates weeks to months after vaccination is unique, considering studies in vaccinated pups show shedding was only found for 3 weeks or less. Significance of these findings will be discussed.

106P

Will pcr detect antibody-neutralized cdv and cpv-2 virus? do storage conditions affect pcr ct values?

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PCR is currently being used routinely for detection of viral infections and diseases in animal shelters and rescues in order to detect, prevent and/or eliminate disease outbreaks. The present study was designed to determine if Canine Parvovirus-2 (CPV-2) and Canine Distemper Virus (CDV) remain positive by PCR after they have been neutralized by antibody. The present study was also designed to determine what effect collection and storage methods have on PCR detection of these viruses. Results showed that antibody neutralized virus was detected by PCR, even though it was not infectious. Results also suggested that conditions of collection and/or storage did not significantly affect the results for PCR detection of CPV-2 and CDV. Studies like these are critical to interpret PCR results correctly and to understand the limitations of this very useful diagnostic tool.

107P

Nanoparticle delivery of siRNAs into feline cells in vitro

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Feline herpesvirus 1 (FHV) is a cause of severe respiratory disease in cats and chronic infections can result in blindness. Though topical antivirals are routinely used for these chronic cases, the clinical response to these treatments has been poor, likely due to owner noncompliance. As a potentially new therapy, we designed siRNAs to specifically target essential viral genes to inhibit replication. These siRNAs produce a 98% reduction in virus replication in primary feline corneal epithelial cells. However, attempts to deliver the siRNAs topically in vivo with commercially available transfection agents have been unsuccessful. Therefore, we have evaluated nanoparticles to aid siRNA delivery. Three sizes of nanoparticles were evaluated, including a 300nm particle produced from FDA approved chitosan, and 200 and 500 nm nanoparticles, both made from two different biocompatible materials. Complexation of the siRNAs with nanoparticles was evaluated using gel shift assays and delivery into cells was evaluated by flow cytometry, using fluorescently labeled siRNAs. Cellular toxicity was assessed using a cell viability assay. siRNA functionality (ability to degrade FHV mRNAs) was tested by detection of FHV specific proteins on treated cell surfaces by flow cytometry and by real time qRT-PCR detection of FHV DNA polymerase mRNA. The siRNA/nanoparticles were non-toxic to cells, had high siRNA binding, and transfected 82% ±8% (chitosan) and >99% ±0.1% (200 nm) ±0.6% (500 nm) of cells with the fluorescently labeled siRNA in vitro. The siRNAs delivered by the 200nm and 500nm particles were non-functional, likely due to lack of release of siRNAs from the particles following entry into the cells. However, the chitosan-delivered siRNAs produced 48% ±7% reduction of FHV-1 specific proteins on the surface of treated cells and reduced FHV DNA polymerase mRNA by 72% ±5% compared to controls. Work is in progress to adjust the particles for increased siRNA functionality within the cells. Nanoparticle use for siRNA delivery allows for stabilization of siRNAs in a deliverable form and facilitates use of a delivery system to prolong contact time between the siRNA/nanoparticle complexes and the ocular surface.

ORAL ABSTRACTS

BACTERIAL PATHOGENESIS

001

Inhibition of Pseudomonas aeruginosa biofilm formation on a biological wound dressing

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Pseudomonas aeruginosa is a gram negative bacterium implicated in many chronic infections, including skin wounds. This bacterium is capable of forming biofilms, which are protective matrices for the organism that allow it to proliferate and tolerate harsh environments. Bacterial colonization of wounds and wound dressings may lead to improper and delayed healing. We have observed that the amino acid tryptophan inhibits formation of P. aeruginosa biofilms on tissue culture treated microtiter plates. We investigated the use of tryptophan for inhibiting biofilm formation by P. aeruginosa in vitro on a biological wound dressing composed of a silicon film impregnated with nylon coated with porcine collagen. Sterile dressing was aseptically cut with an 8mm biopsy punch and placed into the wells of a 48 well plates. The material was exposed to M63 media with and without 10 mM tryptophan and ~10^5 colony forming units of P. aeruginosa (ATCC 27853). The plate was incubated at 30 C for 48 hours after which the amount of biofilm on the material was measured. The biofilm was quantified using Crystal Violet (0.3% w/v) or a phosphatase linked lectin, which catalyzed the colorimetric decay of P-nitrophenylphosphate to P-nitrophenol. Absorbance was measured at either 595 nm for Crystal Violet or 405 nm for the P-nitrophenol. P. aeruginosa formed a robust biofilm on the wound dressing in absence of treatment. Both quantification methods showed that addition of tryptophan to the growth medium significantly inhibited ($p<0.05$) P. aeruginosa biofilm formation on the wound dressing. Pseudomonas aeruginosa biofilm formation on a biological wound dressing was significantly inhibited by tryptophan. Future studies will include incorporation of tryptophan into novel wound dressings, as an antibiofilm agent, for determining biofilm inhibition in experimental mouse skin wounds infected with Pseudomonas aeruginosa.

002

The role of exopolyphosphatase/ guanosine pentaphosphate phosphohydrolase (ppx/gppa) enzymes of *campylobacter jejuni*

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Campylobacter jejuni is commonly found in the intestinal tract of food animals and a major cause of human bacterial foodborne diarrheal illness with seldom fatal neurological outcomes seen worldwide. The inorganic polyphosphate (poly-P) is a key regulator of stress responses and virulence in many bacterial pathogens including *C. jejuni*. Although several enzymes are implicated in poly-P metabolism, the role of exopolyphosphatases (PPX/GPPA) in poly-P homeostasis and *C. jejuni* pathobiology remains unexplored. The genome of *C. jejuni* possesses 2 putative exopolyphosphatases (*ppx1/gppa* and *ppx2/gppa)* and in this study we generated the deletion mutants (Δ*ppx1,* Δ*ppx2)* and the double knockout mutant *(dkppx)* to investigate the role of PPX/GPPA enzymes in *C. jejuni* response to stress and virulence. The Δ*ppx* mutants (Δ*ppx1*, Δ*ppx2* and *dkppx)* exhibited increased capacity to accumulate poly-P, however only Δ*ppx1* and *dkppx* mutant showed decreased accumulation of ppGpp (an alramone molecule) compared to wildtype indicating bifuntional activity of *ppx1/gppa* gene in *C. jejuni*. Interestingly, the expression of *ppk1* and *spoT* genes, which are interconnected with poly-P and ppGpp homeostasis were up regulated in the both Δ*ppx1* and Δ*ppx2* mutants suggesting a compensatory role in poly-P and ppGpp homeostasis. All the Δ*ppx* mutants were displayed defect in virulence related traits such as motility, survival under nutrient limited condition and invasion and intracellular survival in human epithelial cells. However, only the *dkppx* mutant was defective in biofilm formation and osmotic stress tolerance. Bactericidal assay to determine the role of *ppx/gppa* genes in complement mediated killing indicated that both Δ*ppx1*, and Δ*ppx2* mutant were resistant to human complement mediated killing however, *dkppx* mutant was sensitive. In contrary, the chicken serum did not have any effect on the survival of Δ*ppx* mutants. In all the cases complemented strains (*Cppx1, Cppx2*) restored the phenotypic characters similar to wildtype. In conclusion, our study expands the multi-factorial regulation of poly-P and ppGpp metabolism in *C. jejuni*, may serve as unique model for other bacteria as well.

003

Campylobacter jejuni isolates from calves have A, B and C lipooligosaccharide (LOS) biosynthetic locus classes similar to human Guillain Barré syndrome associated strains

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Campylobacter jejuni, a gram negative zoonotic bacterium, is a frequent cause of human gastroenteritis. The acute neuropathy Guillain-Barré Syndrome (GBS) is a post-infectious polyradiculoneuropathy triggered by *C. jejuni* through molecular mimicry. The LOS of *C. jejuni* can mimic gangliosides enriched on peripheral nerves leading to autoimmunity. Members of a family managing a large Midwest dairy operation reported a history of *C. jejuni* infections that lasted several days and recurred over the course of two years. Because up to 37.7% of dairy cattle have been found to shed *C. jejuni*, we sought to determine whether the calves were the source of the family infections. Fecal samples were obtained from 25 randomly selected calves, 1 dog, and 1 family member and cultured for *C. jejuni*. Human and calf isolates were characterized for LOS biosynthesis loci and by multi-locus sequence typing (MLST). *C. jejuni* was cultured from 15 (60%) of calves and one asymptomatic family member; the dog was negative. *Campylobacter coli* was cultured from 2 (1%) of calves. Some calves had diarrhea, but most were clinically normal. Typing of biosynthetic loci showed that several calf *C. jejuni* isolates fell into LOS classifications A, B and C. LOS classes A-C have been strongly associated with *C. jejuni* strains that cause GBS. The human isolate had an LOS class E, which is associated with enteric disease. Three calves had *C. jejuni* with LOS class E. Thus, preliminary typing results suggest that some calf and human *C. jejuni* isolates have similar characteristics. MLST typing is underway to determine whether these isolates are members of the same phylogenetic lineage, thereby providing additional support for zoonotic transmission. Furthermore, finding multiple LOS classes in isolates from these calves demonstrates that there are diverse *C. jejuni* populations present on this farm. Funded by NIH ERIN CRC grant U19AI090872.

BACTERIAL PATHOGENESIS

004

Distribution of virulence genes in Canadian Haemophilus parasuis strains

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The Gram-negative bacterium *Haemophilus parasuis* is the causative agent of Glässer's disease, a significant problem in swine production throughout the world. To date, 15 serovars of *H. parasuis* have been described, although many isolates are untypable. Some serovars (e.g., 1, 5 and 12) are usually, but not always, more virulent than others. Vaccination with both commercial vaccines (typically containing several serovars) and autogenous bacterins have been used to control *H. parasuis*, but in many cases, protection is not complete and the tremendous strain diversity makes it difficult to control the disease. Unlike many related organisms (e.g., *Actinobacillus pleuropneumoniae*), *H. parasuis* does not possess any obvious virulence factors such as toxins that can be targeted for control strategies. Recently, however, a number of genes have been reported to be associated with virulence. The purpose of this study was to determine the presence of these putative virulence genes (*vtaA*1, HAPS_0254, *nhaC*, *fhuA*, *wbgY*, *capD*, *hsdrR* and *fimB*) in nasal isolates from healthy pigs and isolates from lung and systemic sites that had been used for bacterin production. The occurrence of the above virulence genes in the 15 reference strains was consistent with their reported distribution. Except for serovars 1 and 10, serovars predicted to be most virulent carried 5 to 8 virulence genes whilst reference strains of "low virulence" serovars carried 0 or 1 virulence genes. Of 44 bacterin strains examined ~43% had 5 to 8 virulence genes while the remainder carried 0 or 1; a similar bimodal distribution of virulence genes was observed in nasal isolates from healthy animals. Bacterin strains belonging to serovars 5, 13, and 14 (high virulence potential serovars) carried 5 to 8 genes while ~ half of the serovar 4 (moderate virulence) isolates carried 1 virulence gene and the remainder carried 5 to 7 genes; all of the untypable strains had only 1 or 2 virulence genes. Taken together, these data suggest that there is no obvious gene target for the identification of *H. parasuis* strains likely to cause Glässer's disease.

005

Evaluation of invasion by nonpathogenic *Salmonella enterica* serovar Kentucky in poultry intestinal epithelia cells.

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Poultry is a natural reservoir harboring *Salmonella* but is typically asymptomatic upon arrival to processing plants. Due to the continuous risk for cross-contamination among carcasses during processing and with other food products during transportation and handling, a primary concern of the poultry industry is the reduction of *Salmonella* prevalence in the production processing continuum. One option to meet this concern is to decrease the front-load number of bacteria entering the processing continuum. Disruption of the initial colonization in poultry by *Salmonella* could be an effective strategy. The type III secretion system (T3SS) acts as the primary mechanism by which *Salmonella* is able to cross the epithelial barrier and invade the gastrointestinal tract. However, recent literature has emerged suggesting that nonpathogenic *Salmonella* (without a functioning T3SS) are able to actively invade the host intestinal epithelial barrier. The overall objective of this research is to evaluate the ability of nonpathogenic *Salmonella* to invade poultry B5 small intestinal epithelia cells and to analyze the genetic mechanism(s) enabling this process. A bioluminescent reporting system was constructed for real-time monitoring of *Salmonella* during invasion. The pathogenicity of *S. enterica* serovar Kentucky (*S.* Kentucky) was attenuated through the creation of T3SS deletion mutants. Preliminary results demonstrate a stable bioluminescent reporting system based upon the transposition of *lux*CDABE operon into the chromosome of *S.* Kentucky. The structural and effector genes of T3SS located in Salmonella Pathogenicity Islands 1 and 2 were deleted using PCR products in conjunction with λ Red recombinase to render Kentucky nonpathogenic. Future direction of this research is to create a model to efficiently evaluate the phenotypic effects of genetic manipulations in *Salmonella*.

006

Comparative transcriptome analysis using RNA-seq reveals differences in global gene expression profiles between high-pahtogenic and low-pathogenic *Salmonella* Enteritidis strains

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Purpose: Salmonella Enteritidis (SE) is the number one cause of bacterial food-borne gastroenteritis world-wide. Contaminated poultry products such as meat and eggs are the primary sources of human infection. S. Enteritidis is considered as genetically homogeneous serotype; however field strains differ markedly in their phenotype and virulence characteristics in chickens and mice. . The objective of this study was to elucidate the differences in the global gene expression patterns between multiple high-pathogenic and low-pathogenic strains of S. Enteritidis.
Methods: S. Enteritidis strains included in this study were previously characterized as high-pathogenic (HP) or low-pathogenic (LP) based on their virulence in mice and chicken model system. We used the RNA-seq approach to compare the transcriptomes of HP (n=3) and LP (n=3) strains of S. Enteritidis under conditions that mimic the intestinal and intracellular environment.The data analysis was performed using CLC Genomics workbench (CLC Bio, MA)
Results: Comparative transcriptome analysis using RNA-seq identified significant differences in the global gene expression profiles between HP and LP strains. In addition to the genes encoding known virulence factors (eg., SPI-1) several genes encoding metabolic functions and genes with putative or no previously assigned functions (hypothetical genes) were differentially expressed in LP strains as compared with the HP strains under conditions that mimic intestinal or intracellular environment.
Conclusions: This study demonstrates the unique differences in the transcriptome between high and low pathogenic strains of S. Enteritidis and suggests that many uncharacterized regions of the genomes including hypothetical genes or open reading frames with no known functions are differentially expressed. Increased understanding of the specific biochemical and pathophysiological roles of these components will improve our understanding of pathogenesis of S. Enteritidis infection in both human and chickens, a primary reservoir of this serotype.

BACTERIAL PATHOGENESIS

007

Sequence of two plasmids from Clostridium perfringens chicken necrotic enteritis isolates and comparison with C. perfringens conjugative plasmids

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Certain strains of type A Clostridium perfringens isolates cause necrotic enteritis (NE) in broiler chickens, a common infection. A major breakthrough in understanding virulence in NE isolates of C. perfringens was the demonstration that a novel toxin, NetB, is critical for development of the disease. NetB and cpb2 were found on different plasmids. In this study, a netB-positive (pNetB-NE10) and a cpb2-positive plasmid (pCpb2-CP1) obtained from NE isolates were completely sequenced. Both plasmids possessed the large conjugative region characteristic of C. perfringens conjugative plasmids. Comparative genomic analysis of nine C. perfringens conjugative plasmids, including the two plasmids described here, showed extensive gene rearrangements including pathogenicity locus and accessory gene insertions around rather than within the backbone region. The pattern that emerges from this analysis is that the major toxin-containing regions of the variety of virulence-associated C. perfringens conjugative plasmids are organized as complex pathogenicity loci. Analysis of the replication-partition region suggests that this region controls plasmid incompatibility, and that C. perfringens conjugative plasmids can be grouped into at least four incompatibility groups.

008

Comparative genome analysis of an avirulent and two virulent strains of avian *Pasteurella multocida*

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It has been over eleven years since the publication of the complete genome sequence of *Pasteurella multocida* strain Pm70. While the Pm70 genome sequence has been a great asset in our studies, progress has been modest in the identification and understanding of the mechanisms of *P. multocida* virulence. The Pm70 strain was isolated from the oviduct of a layer chicken in 1976 from Texas and is not particularly virulent; it does not cause experimental fowl cholera disease in chickens. By contrast, both the X73 and the P1059 strains of *P. multocida* are highly virulent to chickens and turkeys. Information is sparse on the location and characterization of the genes responsible for differences in virulence of avian *P. multocida* strains. To that end, we undertook a detailed comparative genome analysis of two virulent strains (X73 and P1059) and an avirulent (Pm70) strain of *P. multocida*. This analyses revealed a number of novel metabolic systems and putative adhesins that might explain differences in virulence potential among *P. multocida* strains. Among the unique genes identified in the virulent isolates were those encoding an L-fucose utilization system, and a novel filamentous hemagglutinin named PfhB4. We believe that this work enables future efforts to characterize these unique genes present in the pathogenic strains and determine their roles in virulence and fitness in the avian host.

009

Host specificity in *Pasteurella multocida*

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The bacterium *Pasteurella multocida* is a versatile pathogen and continues to be responsible for a high level of global mortality and morbidity, causing a spectrum of diseases such as a respiratory disease in many animals, including cattle, sheep and goats; a septicemic disease in poultry and wild birds called fowl cholera; and haemorrhagic septicemia in cattle in less developed countries such as in South and Southeast Asia, and sub-Saharan Africa. There has been continued debate over whether the same strains of *P. multocida* can cause infections in more than one species of food animals, or if strains are host-adapted and host-restricted in their disease-causing ability. Rhodes and Rimler in 1993 compared the avian *P. multocida* strain P1059 with the bovine strain P1062 for pathogenicity in turkeys, and found that only the P1059 strain was pathogenic to turkeys. Our work showed that P1062 induced hallmark pathological lesions in a model of bovine pneumonic pasteurellosis, while P1059 did not induce the disease in calves. However, both strains belong to the A:3 serotype. In an effort to better understand host adaptation and host specificity in *P. multocida*, draft sequences of P1059 and P1062 were generated and a comprehensive comparison of all sequenced *P. multocida* strains was performed. A number of insertions/deletions were identified among the strains examined, and greater than 40,000 single nucleotide polymorphisms were identified among these same strains. SNP analysis revealed strong diversifying selection of numerous membrane-associated proteins. Overall, our results suggest a number of candidate genomic changes that might be attributed to adaptation to avian, bovine, or swine species.

010

Roles of type IV secretion system in obligatory intracellular infection.

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Anaplasma phagocytophilum and *Ehrlichia chaffeensis* are obligatory intracellular bacteria that cause emerging tick-borne zoonosis. The type IV secretion (T4S) system is one of important bacterial secretion systems that transfer bacterial proteins and/or DNA into the eukaryotic cells. Our objective of the study is to understand functions of T4S system in these bacteria. Two T4S effectors, an ankyrin repeat-rich protein A (AnkA) and *Anaplasma* translocated substrate 1 (Ats-1) have been identified in *Anaplasma*. AnkA is essential for infection and the major tyrosine-phosphorylated protein in the infected cells. Ats-1 is imported into the mitochondrial matrix and delay mitochondria-mediated host cell apoptosis to benefit *Anaplasma*. We recently found another important function of Ats-1, that is to induce autophagy by binding to Beclin 1, a critical regulator for autophagy. Ats-1 nucleated autophagosomes. Overexpression of Ats-1 in the host cytoplasm enhanced *Anaplasma* infection, whereas cytoplasmic delivery of anti-Ats-1 or Beclin 1 depletion by siRNA suppresses the infection. *beclin 1* heterozygous-deficient mice are resistant to *Anaplasma* infection. Furthermore, *Anaplasma* growth arrest by autophagy inhibitor, 3-methyladenine is alleviated by amino acid supplementation. This suggests that Ats-1 hijacks Beclin-1-dependent autophagy initiation pathway to provide nutrients for bacterial growth. For *Ehrlichia* three candidates for T4S effectors were identified. One of which ECH0825 was also imported by mitochondria and inhibited

010 (continued)

mitochondria-mediated apoptosis. Mitochondrial manganese superoxide dismutase (MnSOD) was increased and the amount of reactive oxygen species (ROS)was reduced in *Ehrlichia*-infected or ECH0825-transfected cells than in control cells. These data suggest that, by upregulating MnSOD, ECH0825 prevents ROS-induced cellular damage and apoptosis to allow intracellular infection. Further studies on functions of T4S effector molecules will undoubtedly advance our understanding of the complex interplay between obligatory intracellular pathogens and their hosts, and may unveil the novel target for chemotherapy.

011

Evaluation of bovine neutrophil activation by *Leptospira*

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Early studies with human innate immune cells (macrophages and polymorphonuclear neutrophils (PMN)) showed that some pathogenic leptospira are efficiently phagoctyosed and killed. However, these studies are lacking in bovines, which can be chronically infected with host-adapted leptospira strains and can become reservoirs of disease. To evaluate the response of bovine PMNs to the presence of pathogenic (*L. interrogans* serovar Pomona strain RM211, *L. interrogans* serovar Copenhageni strain Fiocruz L1-130), host-adapted (*L. borgpetersenii* serovar Hardjo strain JB197 and strain 203) and saprophytic leptospira (*L. biflexa* strain Patoc I) strains, PMNs were isolated from bovine whole blood. After incubation of PMNs with leptospira, various assays measuring neutrophil activation (NET formation, MPO accumulation, cytokine expression and bacterial killing) were performed. Neutrophil extracellular traps or NETs are formed in response to microbial pathogens and have been shown to ensnare these pathogens and inactivate or kill them. Leptospiral species, including heat killed and non-pathogenic saprophytic strain, induced NET formation as measured both by visual examination of cells adhered to microscope slides and assays for quantification of extracellular DNA and increased myeloperoxidase accumulation in cultured cells. Leptospiral antigen could be observed in association with NET-like formations, however, many intact leptospira were also observed not in association with cells or NETs. Limiting dilution culture of PMN-incubated leptospira showed no reduction in viable cell numbers, including the saprophytic strain. In contrast to earlier studies with human cells, bovine PMNs, while activated by the presence of leptospira, are not effective in killing the leptospira. Further studies on the difference in innate immunity between species will lead to better infection models, treatments and preventive measures for leptospirosis.

012

Lymphocyte subpopulations influence murine susceptibility to the agent of epizootic bovine abortion.

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Epizootic bovine abortion (EBA; foothill abortion) is a tick-borne disease responsible for significant fetal losses in California, Nevada and Oregon. The etiologic agent (aoEBA), an intracellular pathogen in the order Myxococcales, has been propagated in mice with severe combined immunodeficiency (SCID), but not immune competent mice. In this study, C57BL/6 (BL6) mice with targeted deletions were infected with aoEBA to better elucidate the role of lymphocyte subpopulations in aoEBA infection. Deletions included TCR $\beta^{-/-}\delta^{-/-}$ (T cell KOs), TCR $\beta^{-/-}$ (αβ T cell KOs), TCR $\delta^{-/-}$ (γδ T cell KOs), Igh-$6^{-/-}$ (B cell KOs) and IFN$\gamma^{-/-}$ (IFNγ KOs). C3H-*scid* and BL6-*scid* mice served as infection controls. Necropsy tissues were probed using either aoEBA-specific IFA and/or PCR to confirm infection status. Infected SCID mice become cachectic and require euthanasia. C3H-*scid* mice appeared more susceptible to infection as compared to their BL6 counterparts. A greater proportion of aoEBA-challenged C3H-*scid* mice were infected (75-100%) as compared to BL6-*scids* (25-75%); C3H mice also developed disease in a shorter period of time ($p<0.05$). This finding would be consistent with the literature describing the BL6 mouse as being relatively resistant to intracellular pathogens. Mice with αβ T cells (γδ T cell KOs and B cell KOs) appeared resistant to infection; growth rates were similar to uninfected counterparts with no signs of disease nor detectable aoEBA. In contrast, mice lacking αβ T cells (T cell KOs and αβ T cell KOs) were susceptible to infection, becoming cachectic; aoEBA was detected in all diseased mice. Mice lacking IFNγ were challenged to further elucidate the specific mechanism by which αβ T cells influence aoEBA susceptibility. All infected IFNγ KOs became cachectic but also developed neurologic signs not noted in other strains. aoEBA was found primarily in the brain of this infected strain. Data suggests that functioning αβ T cells along with INFγ production play a critical role in combating an aoEBA infection. While an infected bovine fetus produces high levels of IgG, this study would support a primary role for T_H1 cells in bacterial clearance.

013

Challenge study to assess association between Moraxella bovoculi and Infectious bovine Keratoconjunctivitis in calves

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The purpose of this study was to evaluate if Moraxella bovoculi can produce clinical signs consistent with infectious bovine keratoconjunctivitis (IBK) in a corneal scarification disease model in calves.
Eight to twelve week-old dairy calves were obtained and housed in individual pens so no nose-to-nose contact between calves occurred throughout the study. Twelve animals per group were required to obtain 80% power to detect a 60% difference in IBK risk between groups based on an expected 10% IBK risk in controls and at least 70% IBK risk in inoculated animals. Thus, our aim was enroll 36 animals in three groups. A 3-arm block-randomized and blinded controlled challenge study was designed as follows: corneal scarification only, corneal scarification and inoculation with Moraxella bovoculi (ATCC strain: BAA- 1259; Origin: California) and corneal scarification and inoculation with Moraxella bovis (strain Epp63-300; Dr. Rosenbusch lab; Origin: NADC). The study was conducted in 3 replicates of 10-12 animals each. Calves were enrolled on day 1 after an ophthalmologist confirmed the absence of any ocular lesions including conjunctivitis. On day 4, calves were scarified and inoculated in one randomly assigned eye. After scarification, calves were observed for corneal lesions until day 18 when they were euthanized. A lesion consistent with IBK lesion was defined as a centrally located corneal ulceration and was assessed by personnel with extensive clinical pinkeye experience. IACUC approval number BC Log #:11-D-0017-A
Of 36 animals purchased for the study, 5 were excluded prior to enrollment due to irregular eyes. Thirty-one enrolled calves were randomly allocated to group. Of the enrolled calves, 9/10 (90%) of M. bovis calves, 0/10 (0%) of M. bovocculi calves and 1/11 (9%) of control calves

013 (continued)
developed visible IBK lesions. All lesions developed in scarified eyes.
The absence of IBK in M. bovoculi BAA-1259 inoculated calves suggests it is not causal organism for IBK in this model and the pathogenicity of this ATCC strain has not been established. The lesion (90%) occurrence in M. bovis demonstrates the efficacy of the model to induced IBK lesions.

014
Comparison of induced small animal models for Guillain Barré syndrome (GBS) as post infectious sequelae to *Campylobacter jejuni* infection
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Purpose: Incidence of gastroenteritis and post infectious sequelae due to *C. jejuni* remains high worldwide. GBS is the world's leading cause of acute neuromuscular paralysis with incidence of 1.3 cases per 1000 in the US annually. GBS disease begins 2-3 wks after *C. jejuni* infection; weakness of limbs develops rapidly followed by symmetrical ascending paralysis. Molecular mimicry between GBS-associated *C. jejuni* lipo-oligosaccharides (LOS) and peripheral nerve gangliosides is thought to mediate demyelination of motor and sensory nerves. However, lack of good, tractable small animal models has limited understanding of GBS pathogenesis and new therapies. Although 33% of chickens orally infected with *C. jejuni* GBS patient strains acquire a form of peripheral neuropathy, chickens are outbred and very different physiologically from humans. We hypothesized that GBS can result in NOD mice or their congenic IL-10 and B7-2 knockouts secondary to *C. jejuni* infection. Methods: Mice were infected with *C. jejuni* strains HB93-13 and 260.94 from GBS patients and examined daily for clinical signs of campylobacterosis and tested weekly for neurological phenotypes through a series of tests such as rotarod, open field testing and DigiGait gait analysis. Autoantibodies to GM1 and GD2a gangliosides in serum were assayed by ELISA and nerves were removed at necropsy, fixed, and stained with hematoxylin and eosin to detect nerve lesions and Luxol Fast Blue Stain to detect myelin damage. Results: Results showed that antiganglioside antibodies and neurological disease develop in NOD WT, NOD B7-2$^{-/-}$, and NOD IL-10$^{-/-}$ mice subsequent to *C. jejuni* infection with GBS patient strains. Mice developed neurological signs including decreased activity, failure to rear, splayed feet, shortened stride length, foot drag, and hind limb paralysis among other signs. Sciatic nerve lesions were consistent with acute inflammatory demyelinating polyradiculoneuropathy (AIDP) seen in GBS. Conclusions: NOD, NOD B7-2$^{-/-}$, and NOD IL-10$^{-/-}$ mice can serve as models for autoimmune neuropathies in humans. Funded by NIH ERIN CRC grant U19AI090872 and contract number N01-AI-30058.

015
Cellulutis in turkeys and the role of gut integrity
A.J. Thachil, K.V. Nagaraja; Veterinary and Biomedical Sciences, University of Minnesota, Saint Paul, MN, USA.

Turkey Cellulitis continued to cause significant economic losses in the US turkey production in USA. *Clostridium perfringens* and *Clostridium septicum* has been consistently isolated from cases of cellulitis in turkeys. It was conceived that loss of gut integrity enables Clostridia to pass the gut barrier and causes cellulitis in turkeys. The objective of our study was to investigate the role of gut integrity in development of cellulitis in turkeys due to *Clostridium perfringens* and *Clostridium septicum* during coccidial infection. Twelve-week old turkey poults were exposed to infective doses of coccidial occysts orally. The birds with and with out coccidial infection were exposed to *C. perfringens* or *C. septicum* orally or subcutaneously to investigate development of cellulitis. Birds exposed to *C. perfringens* orally did not show any clinical signs where as birds exposed to *C. perfringens* and coccidia showed signs of diarrhea and necrotic enteritis. Mortality was noticed in few birds exposed to *C. septicum* and coccidia orally but with atypical signs of toxemia and hepatic necrosis. In our experiments, administration of infective doses of coccidia prior to the administration of *Clostridium perfringens* and *Clostridium septicum* orally did not appear to play a role in the development of cellulitis. However, birds exposed to *Clostridium perfringens* and *Clostridium septicum* through subcutaneous route showed classical signs of cellulitis. Our results support more of a subcutaneous route of infection than oral route of infection for cellulitis development in turkeys.

016
Optimization of *in vitro* growth conditions and DNA extraction from T*reponema phagedenis* isolated from bovine digital dermatitis lesions.
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Purpose: Bovine digital dermatitis is a leading cause of lameness in the US dairy industry where it also represents a significant welfare issue. Our group and others have demonstrated that along with a number of other organisms Treponema spp. can be cultured from a large percentage of these lesions. As part of our ongoing effort to better understand the role that Treponema spp. plays in this disease process we are using a combination of culture-dependent and independent methods to better understand the physiology and metabolism of these bacteria. Treponema spp. are fastidious and their growth in broth media has traditionally been difficult, thus hindering the ability to grow sufficient organisms for DNA purification to be used in downstream sequencing or genetic modification. This study details the systematic evaluation of different culture conditions on the growth and replication of T. phagedenis isolated from digital dermatitis lesions of cattle. Methods: The impact of oxygen concentration (anaerobic, microaerophilic, aerobic), inclusion of fetal calf serum (5,10,15%), shaking, and incubation temperature were tested in broth culture of the organism. Evaluation of bacterial viability and quantification of growth was assessed with flow cytometry using the LIVE/DEAD BacLight Bacterial Viability and Counting Kit. Results: Under optimal growth conditions, broth cultures reached yields greater than 1.0 X 108 cells per ml in 96 hours. DNA extraction from these cultures was increased through the addition of Proteinase K and the quality of DNA was improved through the use of RNase A. As a result of optimization, yields greater than 4ug of high quality DNA could be obtained for each ml of 96 hour broth culture. Conclusions: The results indicate that modifications to the traditional culture techniques employed for this organism result in enhanced growth and that using these techniques large quantities of high-quality DNA can be obtained in less than 5 days. These findings lay the foundation for an improved ability to cultivate this organism for use in challenge experiments and for the development of improved tools for genetic manipulation of this organism.

BACTERIAL PATHOGENESIS

017

Use of anti-SUAM antibodies in a Passive protection model to prevent Streptococcus uberis mastitis

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Research conducted in our laboratory on the pathogenesis of S. uberis mastitis led to the discovery of a novel virulence factor referred to as S. uberis adhesion molecule (SUAM). Further in vitro research showed that antibodies directed against SUAM inhibited adherence to and internalization of S. uberis into mammary epithelial cells, suggesting that SUAM may be a promising antigen for the control of S. uberis mastitis. To define the effect of anti-SUAM antibodies under in vivo conditions, we conducted a passive protection experiment. For this, S. uberis UT888 opsonized with affinity-purified SUAM antibodies was infused into one uninfected mammary quarter of 10 mastitis-free cows. As a control, S. uberis UT888 incubated with fetal bovine serum was infused into one uninfected mammary quarter of 9 mastitis-free dairy cows. Challenged cows were inspected frequently following challenge. During this period, local and general clinical parameters such as milk and mammary scores were recorded, and microbiological analysis of milk was performed. Mammary glands infused with opsonized S. uberis presented lower local inflammatory reaction, lower somatic cell counts, and lower recovery of S. uberis in milk compared to the control group. At the concentration used, anti-SUAM antibodies reduced the severity of S. uberis mastitis , confirming the protective role of anti-SUAM antibodies against S. uberis mastitis. Animal work included in this study was conducted following protocols approved by The University of Tennessee Institutional Animal Care and Use Committee.

018

Defining the role of SUAM in the pathogenesis of Streptococcus uberis mastitis using a SUAM-negative gene deletion mutant

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Research from our lab led to the identification of the Streptococcus uberis Adhesion Molecule (SUAM). We demonstrated that SUAM was involved in adherence to and internalization of S. uberis into bovine mammary epithelial cells (BMEC). Further research led us to construct a SUAM-negative S. uberis mutant that showed significantly reduced adherence to and internalization into BMEC than the isogenic S. uberis strain. To better define the role of SUAM in the pathogenesis of S. uberis mastitis, we conducted an experimental infection study using S. uberis UT888 and its isogenic SUAM mutant clone. SUAM mutant clone was infused into one uninfected mammary gland of five mastitis-free cows and as control, the wild type isogenic S. uberis strain was infused into one uninfected mammary e quarter of four mastitis-free dairy cows. Cows were evaluated frequently following experimental challenge. During this period, local and general clinical parameters were recorded such as milk and mammy scores and microbiological analysis of milk was performed. Mammary glands infused with the SUAM-negative S. uberis mutant presented lower local inflammatory reaction, lower somatic cell counts, and lower recovery of S. uberis in milk compared to corresponding mammary glands of the control group. Results from this investigation are in agreement with our previous in vitro research and confirm that SUAM plays a central role in the pathogenesis of S. uberis mastitis. Animal work was conducted following protocols approved by The University of Tennessee Institutional Animal Care and Use Committee and the Institutional Biosafety Committee.

019

Transcriptome expression profiles of Streptococcus uberis during bovine mastitis

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Abstract Not Available

020

Next-generation sequencing of Streptococcus uberis UT888 genome facilitates quest for virulence /pathogenic associated gene features

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Abstract Not Available

021

Mechanisms of intrinsic resistance to antimicrobial peptides of Edwardsiella ictaluri and its influence on fish gut inflammation and virulence.

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The genus Edwardsiella comprises a genetically distinct taxon related to other members of the Enterobacteriaceae family. It consists of bacteria differing strongly in their biochemical and physiological features, natural habitats, and pathogenic properties. Intrinsic resistance to the cyclic cationic antimicrobial peptides is a specific property of the genus Edwardsiella. Particularly E. ictaluri, an important pathogen of the catfish (Ictalurus punctatus) aquaculture and the causative agent of a fatal systemic infection, is highly resistant to cationic antimicrobial peptides (CAMP). The E. ictaluri mechanisms of resistance to these cationic antimicrobial peptides are unknown. We hypothesized that the lipopolysaccharide (LPS) of E. ictaluri plays role in both virulence and resistance to antimicrobial peptides. The putative genes related to LPS oligo-polysaccharides (O-PS) synthesis were in-frame deleted. Individual deletions of wibT, gne and ugd abolished synthesis of the O-PS, causing auto-agglutination, rough colonies, biofilm formation and motility defects. Deletion of ugd, the gene that encodes the UDP-Glucose dehydrogenase enzyme, causes sensitivity to CAMPs, indicating that UDP-glucoronic acid and its derivates are related to the CAMP intrinsic

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021 (continued)

resistance. E. ictaluri OP-S mutants showed different levels of attenuation, colonization of lymphoid tissues and immune protection in zebrafish (Danio rerio) and catfish (Ictalurus punctatus). Orally inoculated catfish with O-PS mutant strains presented different degrees of gut inflammation and colonization of lymphoid tissues. Here we conclude that intrinsic resistance to CAMPs is mediated by UDP-Glucose dehydrogenase, which have a pleiotropic effect in E. ictaluri influencing LPS synthesis, motility, agglutination, fish gut inflammation and virulence.

022

Penicillin-binding proteins and cefoxitin in Staphylococcus pseudintermedius and Staphylococcus schleiferi subspecies coagulans

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The increased number of methicillin-resistant (MR) staphylococci reported in recent years is considered to be a worldwide emergent problem. MR is frequently reported in *Staphylococcus pseudintermedius, S. aureus* and *Staphylococcus schleiferi* subspecies *coagulans* isolated from numerous pathologic conditions in dogs. The production of an altered form of penicillin-binding protein (PBP) called PBP2a is the most important mechanism associated with β-lactam resistance in staphylococci. The PBPs of *S. aureus* has been characterized and there are 4 native PBPs. To date, the pattern of PBPs in *S. pseudintermedius* and *S. schleiferi subsp coagulans* has not been well characterized. Previous studies have demonstrated that when the 2008 Clinical and Laboratory Standard Institute guideline for oxacillin (OXA) and cefoxitin (FOX) break points are applied there is a failure to accurately identify MR in both MR *S. pseudintermedius* (MRSP) and MR *S. schleiferi* subsp *coagulans* (MRSC). It was hypothesized that differences in the reaction of MRSP and MRSC to FOX, when compared with MRSA, may be based on variations in the PBPs affinity for FOX. Therefore, the aim of this investigation was to characterize the PBPs in *S. pseudintermedius* and *S. schleiferi subsp coagulans.* To identify the pattern of PBPs in *S. pseudintermedius* and *S. schleiferi* subspecies *coagulans,* bacterial membranes or whole bacteria were incubated with Bocillin-FL (Boc-FL) and separated through SDS-PAGE. Competitive binding assays were done to obtain PBPs binding affinity data for FOX in MRSC and MRSP. A five-band PBP pattern (PBP1, PBP2, PBP3, PBP4 and PBP5) was observed in *S. pseudintermedius,* while no difference was observed when PBPs correspond to methicillin-resistant or methicillin susceptible strains. Regarding *Staphylococcus schleiferi* subsp *coagulans,* a pattern of four heavy-weight PBPs (PBP1, PBP2, PBP3 and PBP4) was detected when the ATCC-49545 (methicillin-susceptible) was examined. In contrast, three heavy weights bands were observed in a MRSC. Results of the competitive binding assay between FOX and Boc-FL demonstrated differences in the binding affinity of canine *Staphylococcus* sp and *S. aureus.*

BIOSAFETY AND BIOSECURITY

023

Carriage probability of avian influenza viruses in wild waterfowl influenced by host and environmental factors

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Wild waterfowl are natural reservoirs of avian influenza viruses (AIV) which are occasionally transmitted to other species, such as humans and poultry. The objective of this study was to assess AIV carriage in wild birds as affected by environmental (e.g., soil type, soil pH, land use/ land cover information,proximity to roads and rivers, temperature, precipitation and drought in the period before birds' harvest) and host factors (sex, age, migration, diet and foraging strategy). We used data on RT-PCR detected AIV carriage in wild birds harvested from 2005-2010 in four wildlife management areas located in the coastal region of Texas. The data had cross-classified structure. The results of univariate mixed effect logistic regression analyses, corrected for multiple tests using FDR, suggested that AIV carriage in waterfowl is associated with the season and month of harvest, bird's age, sex, diet, foraging strategy, and migration, as well as with ambient temperature and precipitation in the period before harvest. The final multivariate mixed effect logistic regression model indicated that a bird's young age and herbivorous diet as well as an inverse weighted average temperature over the past 2.5 months before harvest have protective effect but that a bird's dabbling foraging behavior acts as a risk factor for AIV carriage in waterfowl. Furthermore, the model indicated an interaction between diet and foraging strategy. Classification trees confirmed these findings and indicated strong seasonal differences among birds harvested outside of their migration time window, which, if infected, thus must have acquired the infection within the study area. The logistic regression and classification tree modeling approaches had comparable moderately high predictive performances. Collectively, these findings will aid biosecurity and identification of locations and circumstances with an increased probability of AIV carriage in wild birds.

024

Electronic microarrays for detection and typing of high consequence agents in swine

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Natural, accidental, or intentional introduction of high consequence (HC) livestock pathogens can have devastating effects both on the economy and on the public psychology. Development of rapid multiplexed assays on automated user-friendly instruments can allow for more rapid diagnosis and ability to respond to, and recover from catastrophic diseases affecting the livestock industry. In this study, PCR and associated microarray assays for multiplexed detection of HC swine viruses were developed for a user-friendly electronic microarray platform that utilizes rapid electrophoretically-driven hybridization and automates capture probe printing and microarray processing. The swine assay targets viruses that include foot-and-mouth disease virus (FMDV), swine vesicular disease virus (SVDV), classical swine fever virus (CSFV), African swine fever virus (ASFV), vesicular exanthema of swine virus (VESV) and several differential viruses. Strategies used to design primers and probes for detection and genotyping of CSFV, and to fully automate the diagnostic work flow will be presented.

BIOSAFETY AND BIOSECURITY

025

Serotype reactivity of commercial immunoassays for *Salmonella enterica* identification in experimentally-inoculated equine fecal samples

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Salmonella enterica can have a tremendous impact on the management of animal facilities. Comprehensive screening and rapid detection of *S. enterica* in relevant samples is essential for effective infection control in high-risk horse populations. The objective of this study was to characterize the reactivity of 3 commercially available immunoassays, 2 lateral flow antigen detection systems and 1 ELISA, using multiple isolates of *S. enterica* from a variety of common serotypes recovered from hospitalized patients at the Colorado State University Veterinary Teaching Hospital (CSU-VTH) and their housing environment.

A formal random process was used to select 112 *S. enterica* isolates, collected as part of long-term surveillance at the CSU-VTH, representing the 10 most common serotypes (6 serogroups) in this archive. One gram equine fecal samples were inoculated with 1ml of standardized inoculum (~10^2 cfu per ml) into 9ml tetrathionate broth and incubated at 43°C for 18 hr. Samples were tested in a blinded fashion.

Overall, 95.6% of samples were correctly identified by at least one of these immunoassays. In general, serotype reactivity varied by immunoassay. Lateral flow detection systems had the poorest sensitivity for serotypes Cerro (Group K), Mbandaka (Group C1) and Montevideo (Group C1). The ELISA test showed the best performance across serotypes tested.

The findings of this study demonstrate that the utility of these commercial immunoassays varies by serogroup and serotype. This is critically important to their implementation in clinical practice as their value will be dependent upon the most common serotypes detected in a particular population or region.

026

Environmental survival of Equid Herpesvirus -1.

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Purpose: Equid Herpesvirus -1 (EHV-1) can cause primary respiratory disease in horses, but also sporadic, fatal myelopathy and/or abortion complications. Horizontal transmission occurs via droplet infection and fomites. The duration of viral infectivity in the environment has not been studied. The objective of this study was to determine survival time of an EHV-1 suspension on various surfaces: plastic, fabric, leather, wood shavings and straw. It was hypothesized that contact with some materials and/or exposure to environmental conditions will decrease EHV-1 infectivity.

Methods: Duplicate wells of 12-well plates were fitted as follows: plastic(empty well); fabric; leather; shavings, or straw. We added 100 µL (105 PFU) of an EHV-1 (strain: Ohio 03) suspension to each well. Sets of 5 plates were placed in 3 environments: a refrigerator (4oC), an indoor barn area and an outdoor area (direct sun exposure). Two plates remained in the laboratory to be used as t=0. At t= 3, 12, 24, 36 and 48 hrs one plate from each location was collected. Two mL MEM were added to each well. Contents of a well were transferred into a tube and spun. Then, supernatants were collected and frozen immediately. We determined PFU/sample after thawing. We used repeated-measures ANOVA for statistical analysis. Significance was assumed with $p<0.05$.

Results: PFU counts in samples collected at t=0 upon contact with leather and shavings were significantly lower than to plastic, fabric or straw. At least a 1-log reduction in PFU was noticed for most materials after 3 hrs. Continued reduction in PFU counts after t=3 occurred with outdoor samples.

Conclusions: The prompt PFU-count reduction in t=0 samples upon contact with leather and shavings may be due to immediate viral envelope damage. Environmental factors, temperature changes and sunlight had variable effects on preserving virus infectivity depending on the type of contact material. Although there was significant PFU-count reduction within the first 3 hrs, a substantial risk for viral transmission would remain in vivo. Barrier precautions like foot baths, disposable gowns, head cover and gloves remain important during attempts of EHV-1 outbreak mitigation.

027

Efficacy of Sodium Dodecyl Sulfate and Formic acid inactivation of Caprine Arthritis-Encephalitis virus in vitro

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Purpose: Caprine Arthritis-Encephalitis Virus (CAEV) is a lentivirus that causes arthritis and mastitis in adults and encephalomyelitis in kids. CAEV is considered one of the most economically important diseases in dairy goats worldwide. Traditionally, prevention of CEAV transmission for eradication protocols include removal of kids from infected dams prior to consumption of colostrum, and the administration of heat inactivated colostrum/milk or feeding colostrum replacers. The antimicrobial effects of Sodium Dodecyl Sulfate (SDS) have been demonstrated to be efficacious in inactivation of Human Immunodeficiency virus (HIV-1) in milk and Formic Acid (FA) historically has been used in feeding dairy calves due its antimicrobial properties. Both antimicrobials do not affect passive transfer of immunogical or nutritional components of colostrum and/or milk. The objective of this study was determine the efficacy of SDS and FA in the inactivation of CAEV as an alternate for heat inactivation.

Methods: DMEM (Dulbecco's Modified Eagle Medium) or pooled Colostrum was spiked with CAEV (10^5TCID50), and treated with increasing concentrations of SDS to a final concentration 1%, 0.1%, 0.01% and 0.001%. Pooled Colostrum was spiked with CAEV, then treated with increasing concentrations of formic acid to acidify colostrum to a pH of 3, 4, 4.5, and 5, for 15 or 30 minutes. pH was returned to 7.5 with NaOH. Residual viral particles were enumerated utilizing the virus titration assay on goat synovial membrane cells.

Results: SDS concentration of 1% and 0.1% resulted in 99.99% reduction of the input virus, while a concentration of 0.01% and 0.001% failed to significantly reduce the virus titer. Acidification of spiked colostrum to a pH of 3 and 4 after a 15 and 30 min hold resulted in a 99.99% of reduction of infectious virus particles while acidification colostrum to a pH 4.5 and 5 did not significantly reduce the virus titer.

Conclusions: Acidification of Colostrum to a pH of 4 or less for a minimum of 15 minutes or the addition of SDS concentration of 1%-0.1% results in inactivation of CAEV. Future studies include in vivo efficacy studies.

BIOSAFETY AND BIOSECURITY

028

African Swine Fever: Current Situation and Control Strategy

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African Swine Fever (ASF) is an infectious viral disease that causes high economic losses due to necessity of depopulation of pigs in affected areas, sanitary measures, trade restrictions. The virus (ASFV) is stable in the unprocessed meat products and environment, thus, large territories are at risk due to free movement of people and products. The ASFV does not affect people and animals except wild and domestic pigs. Some ticks can carry the virus for years. Adaptation of the virus by changing into less virulent form would mean the threat of an endemic situation in the area. The disease is endemic in sub-Saharan Africa and Sardinia/Italy. There is no treatment or vaccination for ASF. In case of infection with less virulent ASFV strains, recovered pigs could spread the virus life long. By clinical sympthoms, ASF is very similar to Classical Swine Fever. Methods of laboratory diagnostics are well developed and efficient for identification of ASFV and virus-specific antibodies. Experience of eradication of ASF in Spain suggests serological monitoring of pigs.
In the spring of 2007 the ASF was detected in the Caucasus. Same virus was detected in Georgia, Armenia, Azerbaijan, Russia. The ASFV circulating in the Caucasus is a highly virulent virus. No reduction of virulence was observed since the first outbreak. In the last years, the ASF remained in the southern parts of Russia and appeared occasionally as far as St. Petersburg, Tver, N. Novgorod. Domestic pigs play important role in ASFV spread; they infect the wild boars. The virus circulates in the population of wild boars depending on their density in the area. Occasionally, the disease is spread from wild to domestic pigs. There is no evidence of ticks involvement in the process. Thus, human activity is largely responsible for the disease spread. Despite vigorous sanitary measures, the disease has not been stopped. Control strategy for ASF should consider international and local experience. It involves rapid localization of the disease by trained specialists, setting up buffer zones, serologic monitoring of farms, improvement of diagnostic facilities, training of veterinary personnel, development of information and international collaboration.

029

Complying with U.S. export controls as a life science researcher

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Purpose:This presentation is an overview of Department of Commerce regulations and an explanation of the type of commodities relative to biological science research that might require an export license.
Methods:Many of these items are subject to the Export Administration Regulations (EAR) and are controlled by the Department of Commerce. Some items and technology may require a license for export. Export controls related to biological research include controls on certain biological agents and toxins, genetic elements of certain biological agents and toxins, certain vaccines, certain biological equipment and technology related to biological equipment production, development, and use and to non-fundamental research on certain pathogens. Items controlled include the Select Agents (even attenuated) as well as other agents that the Australia Group (international regime) jointly controls.
Results:Questions as to whether an item will require a license can be addressed through a Commodity Classification. Technology (according to the General Technology Note - Supplement 2 to part 774 of the EAR) related to "development" or "production" of Commerce Control Listed (CCL) listed items as well as "use" technology for listed biological equipment is controlled as well. These controls apply when technology is being exported via overseas training, sharing of laboratory protocols, etc. If technology is to be shared with a foreign worker in the United States, the proper term is deemed export. Note that fundamental research (as defined in the EAR Section 734.8 for CCL listed items) does not require a deemed export license for technology. The effect of pre-publication review on classification of research as fundamental will also be discussed
Conclusions: Familiarity with export control regulations contributes to biosecurity and prevents unintentional violation.

030

Development and implementation of an HSEEP compliant avian influenza response training exercise for zoological personnel.

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The nation’s zoos and aquariums form a unique ecosystem where humans, exotic wildlife, domestic animals, and indigenous wildlife all interact with each other on a daily basis. This interaction plays a beneficial role in global conservation efforts and environmental and biomedical teaching and research. However, movement of people and animals within these ecosystems poses the risk of zoonotic disease introduction and dissemination within human and animal populations both wildlife and domestic. Despite the importance of this human-animal interface, there are limited training and program evaluation opportunities in zoonotic disease outbreak preparedness that target the zoological community and the regulatory agencies that would respond to a zoonotic foreign animal disease event at a zoo. “Flu at the Zoo: A Tabletop Exercise of Avian Influenza Outbreak Response in Zoos and Aquariums” was a USDA-Animal Care funded project whose goal was to enhance the preparedness and communication among zoological personnel in the Illinois, Indiana, and Missouri region to respond to an outbreak of avian influenza in a captive wildlife population. Representatives from all 16 AZA accredited zoos and aquariums in the region participated in the exercise which was held in Bloomington, Illinois on June 6, 2012. A total of 83 participants from 10 states and Washington, D.C. attended the event. As a result of this exercise, the 2009 USDA-APHIS-AZA Management Guidelines for Avian Influenza: Zoological Parks & Exhibitors Outbreak Management Plan version 322 has been updated to reflect findings from the exercise. Recommendations from the After Action Report developed from Flu at the Zoo included the need for increased training opportunities for zoological community personnel in ICS and NIMS. In addition, it was recommended that communications be enhanced between zoos and aquariums and the local, state, and federal agency personnel that would serve as first responders in an FAD event.

COMPANION ANIMAL EPIDEMIOLOGY

031

From licking stamps to clicking buttons - moving from conventional questionnaires to online surveys

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Plenary lecture: Questionnaires have been a key data collection tool in epidemiological research, and a lot of papers and textbooks have addressed the issue how to best design a questionaire and run a survey. In that context, getting the questionnaire to the individuals (animals) in the target population has always been an important methodological consideration. Conventional postal mail surveys were extensively utilized in that context, with response rates averaging around 30%. In our increasingly Internet-connected world it was a natural process to replace the conventional postal mail survey (who still sends and reads real letters anyway) by email- or web-based online surveys. But are they really the same? Do they reach the same target population when compared to postal surveys, and with a similar performances (especially response rate)? What are their main pros and cons? With the experience of several postal and recently also online surveys performed within veterinary epidemiological studies, I will present the technical issues, advantages and challenges - based on own experience and a literature review - related to online-based surveys, and contrast them to the good old times of sending letters and postcard reminders.

032

Nasal shedding of Equine Herpesvirus-1 from horses in an outbreak of Equine Herpes Myeloencephalopathy in Western Canada

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Recently, Equine Herpes Myeloencephalopathy (EHM) was described as a "potentially emerging disease" by the USDA. Absence of information regarding shedding in horses with naturally occurring disease, and knowledge that latent carriage complicates management, makes development of objective quarantine recommendations for managing outbreaks difficult. Objectives of this report were to describe an outbreak of EHM in western Canada during the spring of 2008 and evaluate nasal shedding duration of Equine Herpesvirus - 1 (EHV-1) in horses affected with EHM during this outbreak.
All horses on affected premises were monitored. Those horses developing EHM were sampled in a longitudinal outbreak investigation. Nasal swabs were collected daily from 16 of 20 horses affected by EHM. A qPCR was performed on 98 of 246 nasal swab samples to determine nasal shedding duration. Historical and clinical information was analyzed to evaluate potential risk factors for developing EHM and duration of shedding during this outbreak.
The last day shedding was detected in any horse was Disease Day 9. EHV-1 was detected in two-thirds of horses tested on Disease Days 0-3. The amount of EHV-1 DNA found in nasal swabs varied markedly and was not associated with disease severity or age. The odds of developing EHM were greater for febrile horses (OR = 20.3; 95% CI 3.4-390.3; P = .01) as well as for horses attending the riding clinic (OR = 4.1; 95% CI 0.84-21.65; P = .08).
Based on these findings, in the absence of laboratory testing, we recommend biosecurity measures be implemented when managing EHM cases for a minimum of 14 days beyond the onset of clinical signs. This report illustrates that animal managers cannot rely on the severity of clinical signs to predict the duration of EHV-1 shedding.

033

Risk factors for antimicrobial resistant *Salmonella* spp. and *Escherichia coli* carriage in pet dogs from volunteer households in Ontario (2005-2006)

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The purpose of this study was to determine the pet-related risk factors associated with antimicrobial resistant *Salmonella* spp. and *Escherichia coli* (*E. coli*) carriage in the feces of pet dogs from volunteer households in Ontario, Canada. From October 2005 until May 2006, 138 dogs from 84 households in Ontario were recruited to participate in a cross-sectional study. Five consecutive daily fecal samples were collected from each dog and cultured for *Salmonella* spp. and *E. coli*. In total, 515 bacterial isolates from 136 dogs from 83 households were collected and sent for antimicrobial susceptibility testing. Multilevel logistic regression models with random effects for household and dog were created to identify pet-related management factors associated with general antimicrobial resistance (AMR), multiclass resistance, and ampicillin resistance. Factors investigated included bacterial species, type of diet fed, veterinary treatments, and other pet demographics. Approximately 19.6% of the isolates tested were resistant to at least one antimicrobial, and 11.3% of the isolates were resistant to ≥2 classes of antimicrobial ("multiclass" resistance). From the multilevel models, bacterial species (*Salmonella* vs *E. coli*), being fed a homemade diet or having any homemade food added to dog's diet, being fed a raw diet or having anything raw added to a dog's diet, being fed a homemade raw food diet, and being fed raw chicken in the past week were statistically significant risk factors for general antimicrobial resistance in this population of dogs. Being given an herbal product (e.g., glucosamine) was a significant risk factor for multiclass and ampicillin resistance. Being vaccinated annually and being treated with heartworm medication in the previous six months were found to be sparing factors for general resistance. These results may suggest that compliance to veterinary recommendations concerning preventive medicine and diet may reduce exposure to antimicrobial resistant bacteria. These results also highlight the potential public health risk of including raw animal products in canine diets.

COMPANION ANIMAL EPIDEMIOLOGY

034

Syndromic surveillance for nosocomial infections in small animal veterinary referral hospitals

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Purpose:To design a standardized syndromic surveillance system for use in multiple small animal critical care referral hospitals in the United States in order to estimate rates of occurrence of common nosocomial events among patients considered to be at higher than average risk for developing disease.
Methods:This multicenter prospective longitudinal study included weaned dog and cat patients (n=1951) that were hospitalized in the critical care unit of one of 4 participating veterinary referral hospitals during a 12 week period in 2006. At the time of patient discharge a survey form was filled out by the primary clinician that included basic patient demographics and information about procedures and treatments the patient received. The clinician was asked to give their best clinical impression about whether specifically defined nosocomial syndromes were recognized during hospitalization. Data were analyzed to identify risk factors associated with nosocomial events.
Results:Controlling for hospital of admission, 16.3% of dogs (95% CI, 14.3 to 18.5) and 12% of cats (95% CI, 9.3 to 15.5) were reported to have had a nosocomial event occur during hospitalization. Risk factors found to have a positive association with the development of a nosocomial event were longer hospital stays, placement of a urinary catheter, surgical procedures being performed, and the administration of anti-ulcer medications and antimicrobial drugs excluding those given peri-operatively.
Conclusions:Syndromic surveillance systems can be successfully standardized for use across multiple hospitals in order to effectively collect data pertinent to nosocomial event rates and risk factors for occurrence.

035

Survey to investigate pet ownership and attitudes to pet care in metropolitan Chicago dog and/or cat owners.

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Purpose: Creating regionally sensitive pet evaluation matrices is fundamental to establishing practical, locally relevant shelter protocols. Our primary aim was to investigate trends in average dog and cat standard of care and acquisition among residents of Chicago and a secondary aim was to compare the attitudes and typical standards of care provided by owners of shelter-acquired pets with those of residents who acquired their pets from other sources.
Methods: We collected data from 529 in-person surveys administered in 5 Chicagoland (zip codes 60600-60660) petstore locations, representing 582 dogs and 402 cats.
Results: The typical dog owner owned an average of 1.3 ‘indoor’ dogs acquired from a breeder (36%) or shelter (33%); and would spend >$1000 for veterinary care of a ‘treatable’ (77%) condition. When comparing owners of shelter-acquired dogs and owners of dogs obtained from friends/family/neighbors, owners of shelter-acquired dogs were significantly more likely to spend >$1,000 on ‘treatable’ (P=0.0123) and ‘manageable’ (P=0.0005) condition care.
Similarly, the majority of cat owners acquired their cat(s) from shelters (55%); characterized their pet(s) as ‘indoor’ (84%); although cat owners tended to own more cats (1.8). Average cat owners were significantly less likely to pay >$1000 for a ‘treatable’ condition (62%, P=0.0002). Furthermore, the average cat owners were less likely to bring their cat(s) to a veterinarian for vaccinations (88%, P<0.0001) or an annual exam (71%%, P<0.0001) when compared to average dog owners (99% and 93%, respectively). When comparing shelter acquired cats to those from friends/family/neighbors, cats acquired from shelters were significantly more likely to have an owner who was willing to spend >$1,000 on ‘treatable’ (P=0.0349) and ‘manageable’ (P=0.0433) condition care. Shelter-acquired cats were significantly more likely to receive vaccinations (P=0.0388) and annual exam (P=0.0443) compared to cats obtained as strays.
Conclusions: Understanding the attitudes and standard of care of average Chicago pet owners across various forms of acquisition is critical to creating successful strategies to increase local shelter adoption rates.

036

Birth and death rate estimates and selected owner demographic data associated with cat, dog, pet bird, and horse ownership in U.S. households in 2006

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Purpose: Birth and death rate estimates for cats and dogs in U.S. households (HH) are useful to veterinarians, manufacturers of pet products, pharmaceutical companies, animal shelter and animal control officials, and others. Methods: Using data from the 2006 AVMA survey of pet owners, we estimated national birth and death rates which did not differ significantly from our previously published estimates based on 1996 data. Results: When these rates were stratified by U.S. Census Bureau divisions and selected owner demographic variables, significant differences were found. Some parts of the country had significantly higher birth rates defined, for example, as the number of kittens born per 100 cats in HH. Birth and death rates for cats and dogs were inversely associated with HH income. Significant differences in birth rates for cats and dogs were also found to be associated with education, employment status, and community size. Selected data on the birth and death rates of pet birds and horses were also estimated.
Conclusions: These rates provide insight into the problems of the companion cat and dog surplus which can have potential public health and safety impacts as well as environmental effects.

COMPANION ANIMAL EPIDEMIOLOGY

037

Use of survival analysis to assess the effects of fee structure on post-adoption relinquishment of dogs and cats

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The purpose of this study was to determine the effect of adoption fee amount on the hazard of post-adoption relinquishment, while controlling for other factors such as: species (dog vs cat), age, sex, neuter status (at adoption), breed, and unknown shared frailty factors associated with individual shelters.
Records came from a large database (PetPoint™) of shelter adoptions in the U.S.A. We extracted demographic information on each animal, including adoption and return date. The data indicating the length of time animals remained adopted were highly right-censored and were recorded as: relinquished (1) or not relinquished (0). The sex, neuter status, and breed were coded as indicator variables. As age was a time-dependent variable, we included adoption age (<6 months, >6 months) and also age when either the study ended or the animal was relinquished. Dogs and cats were analyzed separately. We created ordinal variables for adoption fees. Cox proportional hazard regression was used to estimate survival function and hazard ratios associated with fee and other covariates for cats and dogs, while adjusting for unknown shelter frailties.
Our analysis included results from 128,449 cats and 161,666 dogs. Of these, 8,776 cats were returned, and 20,671 dogs were returned through June 1, 2011. Overall, dogs and cats differed greatly in post-adoption relinquishment ($P < 0.0001$). The adjusted hazards associated with adoption fees for both cats and dogs were significantly different. Importantly, the hazards were not constant overtime, but decreased rapidly during the initial post-adoption period and then became nearly constant past 6 months post adoption.
In conclusion, dogs were much more likely to be relinquished post-adoption than cats. Generally, the lower the adoption fee, the more likely and more rapidly adoption relinquishment occurred. Neutered dogs (at time of adoption) were more likely to be returned than non-neutered animals; however, for cats it was the opposite. The first 100 days after adoption appeared to be very critical for predicting animal adoption relinquishment. After 100 days, the hazard rates for returning animals stabilized at very low levels.

038

Risk factors for the development of malignant histiocytosis in Bernese Mountain Dogs

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Purpose:Bernese Mountain Dogs are at an increased risk of developing malignant histiocytosis when compared to other breeds. In order to elucidate which exposure variables affect the outcome of malignant histiocytosis in Bernese Mountain Dogs the influence of genetics must be accounted for. The purpose of this study was to examine multiple factors that may be associated with the outcome of malignant histiocytosis while taking the genetic influences into account. Methods:The study was conducted as a cross-sectional survey. The eligible study population consisted of Bernese Mountain Dogs registered with the Berner-Garde Foundation. Owners who elected to participate were invited to complete an electronic survey. Mixed effects logistic regression and conditional logistic regression were used in parallel to examine associations between potential risk factors (exposure variables) and the occurrence of malignant histiocytosis.
Results:Data were collected for a total of 216 Bernese Mountain Dogs, representing 140 different litters. When controlling for litter of origin (as a surrogate for genetics), dogs diagnosed with orthopedic conditions were 2.5 times more likely to develop malignant histiocytosis. These data also show that dogs receiving long-term medications are actually at a considerably lower risk of developing malignant histiocytosis than are dogs that do not receive long-term medications. The most common medications reported by owners to have been used long-term for their dogs included anti-inflammatory medications.
Conclusions:The results of this study suggest the use of anti-inflammatory medication may help decrease the risk of developing malignant histiocytosis in Bernese Mountain Dogs. More research in this area is warranted.

039Prevalence of feline influenza virus infection in cats in Bangladesh.

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The cat has been living in close association with human for at least 3500 years, the ancient Egyptians routinely used cats to keep mice and other rodents away from their grain. The history of domestic cat may stretch back even further, as 8,000 year-old bone of humans and cats were found buried together on the island of Cyprus. Currently, the cat is the world's most popular household pet. Feline influenza (cat flu) is an infectious disease that affects the upper respiratory tract and symptoms usually appear within 1 to 3 days.It is caused by a group of RNA viruses under the family Orthomyxoviridae. It infects humans, other mammals, and birds, and causes all flu pandemics. This study was carried out to determine the prevalence of feline influenza virus in cats in Bangladesh using RapiGen® Feline Influenza Virus Antigen (FInV Ag) test kit. The overall prevalence of feline influenza virus in cat in Mymensingh Sadar upazilla in Mymensingh district and Bholahat upazilla in Chapai Nawabgonj district of Bangladesh was 3.28%. Prevalence of feline influenza virus was 3.45% and 2.86% in street and pet cat, respectively. The prevalence of feline influenza virus infection was 3.75% in <1 years age group, 5.00% in >7years age group and no positive was found in 1-7years age group. The prevalence of feline influenza virus was 18.18% in sick cat with clinical sign of conjunctivitis and labored breathing and no positive was found in apparently healthy cat. To the best of knowledge this reported first time to detect the prevalence of feline influenza virus infection in cats in Bangladesh.

040

The reliability of a survey to score cat socialization from unsocialized to highly socialized

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COMPANION ANIMAL EPIDEMIOLOGY

040 (continued)
Purpose: Animal shelters and rescue groups make decisions about whether cats are socialized or unsocialized on a daily basis. However, no valid methods of determining cats' socialization status exist.
Methods: A survey (CS) was designed for cat owners and caregivers to report cats' behaviors towards people in their normal environments. Respondents rated cats' behaviors in various situations from 0 (never) to 10 (always). An overall socialization score (OSS) was calculated as the median of these ratings. Two caregivers independently completed the CS for each cat (inter-rater reliability) and repeated it one month later for the same cat (test-retest reliability). At one sanctuary 2 caregivers rated 31 cats. At the second, 36 caregivers with 48 unique pairings rated 54 cats (inter-rater) and 48 cats (test-retest).
Results: Spearman correlation coefficients and 95% CIs were calculated by question and OSS. Inter-rater and test-retest agreement was higher at the first sanctuary (0.71-0.98) than the second (0.21-0.85). Inter-rater correlations were <0.5 for a cat's reaction to a new place, reaction to caregiver while cat eats, cat's activity level and cat staying near caregiver. Test-retest correlations were <0.5 for reactions to caregiver while cat eats and cat's response to an unfamiliar person. While OSS was high with the full set of questions (0.71-0.99), maximizing the OSS inter-rater and test-retest correlations by examining individual raters, original question inclusion criteria and factor analyses at both sanctuaries suggested dropping playing with toy and activity level (0.72-0.98).
Conclusions: The CS shows promise as a reliable instrument to assess cats' socialization to humans.

EPIDEMIOLOGY AND ANIMAL HEALTH ECONOMICS

041
Prioritization of zoonoses in North America: A public perspective
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Zoonotic diseases have considerable impact on public and animal health. As resources are limited for the control and prevention of zoonotic diseases, it is necessary to prioritize diseases to direct resources into those with the greatest needs. We used conjoint analysis (CA), a well-established quantitative method in market research, to identify the relative importance of 21 key characteristics of zoonotic diseases that can be used for their prioritization in Canada and the US. Relative importance weights from the CA were used to develop a point-scoring system to derive a recommended list of zoonoses for prioritization in Canada and the US. Six focus groups identified 21 characteristics for determining prioritization; this was used to construct the CA. Over 1,500 participants from the general public were recruited to complete the online survey (761 from Canada and 778 from the US). Hierarchical Bayes multinomial logit models were fitted to the survey data to derive CA-weighted scores. Scores were applied to 62 zoonotic diseases of public health importance in Canada and the US to rank diseases in order of priority. This study is the first to describe a systematic and quantitative approach to the prioritization of zoonoses in North America involving public participants. We found that individuals with no prior knowledge or experience in prioritizing zoonoses were capable of producing meaningful results using CA. More similarities than differences were observed in the strength of preference for disease criteria between countries suggesting general agreement in disease prioritization between Canadians and Americans. We recommend CA as a potential tool for the prioritization of zoonoses. Understanding the perception of the public may offer healthcare professionals the opportunity to improve public education and risk communication.

042
Prioritization of Zoonoses in North America: Animal and human health professionals' perspective
V. Ng, **J.M. Sargeant**; Population Medicine, Ontario Veterinary College, Guelph, ON, Canada.

Purpose: Zoonoses account for 58% to 61% of all communicable diseases causing illness in humans and up to 75% of emerging human pathogens. Although the impact of zoonoses on animal health and public health in North America is significant, there has been no published research on prioritization of zoonoses in this region. This study describes the novel use of a well-established quantitative method, conjoint analysis (CA), to identify the relative importance of 21 key characteristics of zoonotic diseases that can be used for their prioritization in Canada and the US. Relative importance weights from the CA were used to develop a point-scoring system to derive a recommended list of zoonoses for prioritization in Canada and the US. A total of 1,471 participants with a background in epidemiology, public health, medical sciences, veterinary sciences and infectious disease research were recruited to complete the online survey (707 from Canada and 764 from the US). Statistical models were fitted to the survey data to derive CA-weighted scores for disease criteria. Scores were applied to 62 zoonotic diseases to rank diseases in order of priority. We present the first zoonoses prioritization exercise involving public health, veterinary and medical professionals in North America. Our previous study indicated individuals with no prior knowledge or experience in prioritizing zoonoses were capable of producing meaningful results with acceptable model fits. This study suggests professionals with some knowledge or experience in prioritizing zoonoses were also capable of producing meaningful results with better-fitted models than the general public. Despite more similarities in demographics and model fit between the combined public and combined professional groups, there was more uniformity across priority lists between the Canadian public and Canadian professionals and between the US public and US professionals. The priority lists derived from this study will help formulate a framework for policy development for the control and prevention of zoonoses in Canada and the US.

043
Methicillin Resistant *Staphlylococcus aureus* in Dairy Farms - Is there a need to worry?
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Abstract Not Available

EPIDEMIOLOGY AND ANIMAL HEALTH ECONOMICS

044

Non-tuberculous mycobacteria in the pastoral ecosystems of Uganda: "One health, One ecosystem"

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The roles of non-tuberculous mycobacteria (NTM) and Mycobacterium Tuberculosis Complex (MTC) in infections in pastoral ecosystems of Uganda were investigated. NTM are opportunistic pathogens that are acquired from the environment. The occurrence of MTC in cattle and the existence of suitable biotopes for NTM in the water sources in their areas increase the risk of infections to pastoral communities. There was a need to investigate the role of NTM and MTC in causing cattle and human disease in the pastoral areas of Uganda.
Objectives: Phase 1. a) To isolate and characterize Mycobacteria causing tuberculous lesions in slaughter cattle and b) To isolate and characterize the mycobacteria causing cervical lymphadenitis in patients in the same pastoral areas of Uganda. Phase II was -To identify the role of environment as source of NTM infections to cattle and humans.
Methods: Phase 1, 43 isolates from human cervical lymph node biopsies and 61 lessoned organs from slaughter cattle were characterized. In phase two, 48 isolates from 310 environmental samples were studied. The AccuProbe culture identification kits, Spoligotyping and IS1311 and IS1245 -RFLP , INNO-Lipa test and 16S rDNA sequencing were used.
Results: Of the 43 human biopsies, MAC (41.7%), M. intracellulare(8.3%), M. tuberculosis (29.2%),and M. bovis (12.5%) were isolated. Composite dendrograms of IS1311 and IS1245 RFLP showed 3 human and 2 animal isolates were identical. From cattle, M.bovis(51.4%), M. fortuitum perenigrum Complex (16.25%), MAC (13.5%), M. intracellulare (2.5%), gordonae (8.1%) and M. non chromogenicum (5.4%) were isolated. From the environment, NTM were detected from soil -25.3%, water-11.8% and cattle feces-9.1%. M. fortuitum- peregriunum complex was the most dominant in all the 3 ecosystems, followed by MAC. Shared infections included M. bovis (cattle and human), MAC and M. intracellulare (environment, cattle and human).
Conclusion: The isolation of similar MTC in cattle and humans and NTM in cattle, humans and environment confirms existence of shared infection of cattle and humans from the environment in addition to infections from livestock and affirms need for “one health” approach to pastoral health

045

Comparative study of the prevalence of brucellosis in cattle, goats and humans from farms in southwestern Uganda

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Purpose: A cross-sectional study was conducted to determine the seroprevalence of brucellosis in cattle, goats, and humans from 75 cattle farms in two districts of southwestern Uganda, located in areas with known wildlife reservoirs of brucellosis, and different livestock populations and management practices. This is part of a larger study to relate brucellosis transmission with interaction between humans and livestock in areas with high human-livestock contact and high potential for zoonotic disease transmission.
Methods: A total of 75 farms were enrolled. Blood samples were collected from 773 cattle from 75 farms, 322 goats from 73 farms, and 235 humans from 70 farms. Milk samples were collected from 636 cows from 69 herds. Questionnaires were used to collect risk factor data from all 75 farms. Risk factor data for livestock included signalment, and reproductive history, and human data included signalment, health history, consumption of raw milk, and contact with livestock. Farm-level data included livestock and human inventories, health histories, livestock management, and human contact with livestock and wildlife.
Serum and milk samples were taken to local veterinary laboratories and tested according to OIE protocols. Brucellosis was identified using Rose Bengal Plate Test for livestock serum using Brucella abortus and B. melitensis antigens. The Milk Ring Test was conducted on cattle milk. Commercially-available IgM and IgG lateral flow assay kits were used to detect brucellosis from human sera.
Results: The herd prevalence of brucellosis was 90.4%, 35.6% and 32.9% for cattle, goats, and humans, respectively. Individual prevalence in cattle was 31.3% (14.2% by sera, 22.0% by milk), 16.5% in goats, and 11.9% in humans. Prevalences were associated with geographic location, herd sizes, high-risk animal contacts and prevalence in other hosts.
Conclusions: Brucellosis is widespread and prevalent in both livestock and humans in the study areas, and ongoing molecular typing of species and strains of Brucella will make significant contributions to understanding how the dynamic interplay between livestock, humans, and their ecology influence the transmission of zoonotic diseases.

046

Time series model for human and bovine brucellosis cases in South Korea between 2005 and 2010

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Purpose: Brucellosis is considered to be one of the most important zoonotic diseases in the world. In South Korea, most human cases occur from not wearing protective clothes or gloves when in contact with suspected cattle or materials, but the ingestion of cheese and raw milk is very rare. The main objective of this study was to use retrospective case data to develop an appropriate time series model for cattle to human transmission in South Korea.
Methods: The total monthly human and cattle case counts as well as national population data between Jan 2005 and Dec 2010 were obtained for analysis, and the temporal relationship was evaluated using an Autoregressive Integrated Moving Average with exogenous input (ARIMAX).
Results: Cross-correlations between human and cattle cases were evaluated, and the strongest correlation was detected at the lag of 0 and 1 months. In the final model, autoregressive integrated moving average (ARIMA) (0, 1, 1) - autoregressive (AR) (0, 1) provided the best model.
Conclusions: Lags of 0 and 1 month in cattle cases appear to be good statistical predictors of future human case numbers in South Korea. The 0 month lag, presumably related to disease transmission factors, does not however allow for the practical use of this model for public health forecasting.

EPIDEMIOLOGY AND ANIMAL HEALTH ECONOMICS

047

The prevalence and spatial distribution of avian reovirus among Ontario broiler chicken flocks

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Avian reovirus (ARV) is the causal agent of tenosynovitis, also known as viral arthritis, which is an economically significant disease of broiler chickens (meat-type birds) and turkeys. Avian reovirus causes swelling of the hock joint(s) and tendon sheath(s), and in severe cases, rupture of the tendon(s); affected birds have difficulty moving toward feed and water, resulting in poor growth or death. Birds that survive to slaughter might be downgraded because of inflammation in the joint(s). The objective of this study was to determine the prevalence and spatial distribution of ARV among commercial broiler chicken flocks in Ontario.

A cross-sectional study was conducted between July 2010 and January 2012. The total study population was 231 flocks. Each month, an equal number of randomly selected flocks were recruited to account for potential seasonal variation in pathogen prevalence. Samples were collected at six processing plants, which represented 70% of Ontario's broiler processing. At the plants, 15 blood samples, and 15 whole intestines per flock were collected at slaughter and submitted to the Animal Health Laboratory at the University of Guelph for testing; ELISA was used to detect ARV antibodies, and virus isolation in eggs and in cell culture was used to detect viral shedding. A flock was considered to be exposed to ARV if 1) it was positive on virus isolation and the mean titer of ELISA was ≥ 2,000, or 2) it was negative on virus isolation but the mean titer was ≥ 2,000, or 3) it was positive on virus isolation but the mean titer was between 500 and 2,000, or 4) it was negative on virus isolation, and the mean titer was between 500 and 2,000 but at least 1 bird had a titer greater than 2,000. A flock was considered to be non-exposed to ARV if the mean titer was < 500 regardless of the result of virus isolation. Results to date reveal that the prevalence of ARV among Ontario broiler flocks is 0.88 (95% CI 0.82-0.95). The risk of infection by broiler district will be determined using ArcGIS to address current knowledge gaps in the distribution of this pathogen in Canada's largest broiler producing province.

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Prevalence, characterization, and seasonal variation of *Clostridium perfringens* in Ontario broiler chicken flocks.

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Clostridium perfringens is the bacterial agent responsible for necrotic enteritis, a financially devastating disease in broiler chickens. Interest in characterizing this pathogen re-emerged after the discovery of *beta2* and *netB*. There is scarce information on the baseline level, genotypes, and seasonal variation of this pathogen in Ontario broiler chickens. The objectives of this study are to 1) determine the prevalence and genotypes of *C. perfringens* in a representative sample of Ontario broiler chickens flocks; 2) assess the association of *C. perfringens* flock prevalence and season during grow out; and 3) assess the association of *beta2* and *netB* in *C. perfringens* isolates. Three pooled (cecal swabs) samples from 15 birds per flock from 231 flocks were tested for *C. perfringen*s. Polymerase chain reaction (PCR) was used to test for genes encoding the four major toxins, enterotoxin, and *beta2*, and real-time PCR was used to test for *netB*. C*lostridium perfringens* was isolated from 181 of 231 (78.4%) flocks. *NetB* was detected in 71 of 231 (30.7%) flocks. All isolates were type A, except for one type E isolate. *Beta2* and *netB* were detected in 535 (85.1%) and 169 (26.9%) of 629 isolates, respectively. One hundred and fifty-two isolates with *netB* also carried *beta2*. The enterotoxin gene was not detected in any of the isolates. The odds of testing positive for *C. perfringens* without *netB* compared to testing negative for *C. perfringens* were significantly higher in the summer (odds ratio (**OR**) = 5.56, 95% Cl: 1.93 - 15.93, p = 0.001) and fall (OR = 3.06, 95% Cl: 1.12 - 8.32, p = 0.029) than in the winter. Particularly in the summer, the odds of testing positive for *C. perfringens* without *netB* were significantly higher than testing positive for *C. perfringens* with *netB* (OR = 3.36, 95% Cl: 1.30 - 8.70, p = 0.013). An unconditional logistic regression model with a random effect for flock indicated that *netB* is more likely to be present in isolates with *beta2* (OR = 3.97, 95% Cl: 1.46 - 10.78, p = 0.007). A high prevalence of *C. perfringens* type A was expected. The virulent marker, *net*B, was present in an unexpected number of flocks. Strategies to reduce *C. perfringens* in broiler flocks need to consider season in the planning process.

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Prevalence, seasonality, and geographical distribution of chicken anemia virus, fowl adenovirus, and infectious bursal disease virus in Ontario broiler chickens.

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Chicken anemia virus (CAV), fowl adenoviruses (FAdV), and infectious bursal disease virus (IBDV) cause significant economic losses to the poultry industry worldwide. There is paucity of information on the prevalence, seasonality, and geographical distribution of these viruses in healthy broiler flocks in Ontario. Our study aimed to 1) estimate the prevalence of CAV, FAdV, and IBDV in Ontario commercial broiler flocks; 2) assess seasonality and geographical distribution of CAV, FAdV, and IBDV; 3) determine prevalent genotypes of FAdV and IBDV; and 4) determine the association between FAdV and the presence of IBDV, and CAV.

Tissues (15 blood tubes, 15 cecal tonsils, and 15 cloacal swabs per flock) were obtained from 231 broiler flocks and analyzed at the Animal Health Laboratory using Enzyme linked immune-sorbent assay, polymerase chain reaction (PCR) and nucleotide sequencing of PCR products. Associations between viruses, and with broiler districts and seasons, were estimated using logistic regression models (STATA IC 10). Choropleth maps on spatial distribution of the viruses were produced using ArcInfo 10.

The prevalence of CAV and IBDV were 17.7% and 26.4%, respectively. The prevalence of FAdV was 69.3%. District 1 (OR = 3.71; p = 0.033) had a higher prevalence of CAV compared to district 3. District 5 (OR = 6.33; p = 0.006) had a higher prevalence of IBDV compared to district 3. The prevalence of CAV was lower in summer (OR = 0.33; p = 0.041) compared to spring. The prevalence of FAdV was lower in the spring (OR = 0.32; p = 0.024) compared to winter. There was no association between FAdV status and both CAV status (OR = 1.72; p = 0.183) and IBDV status (OR = 1.91; p = 0.066). The genotypes detected were; FAdV-01, FAdV-11, FAdV-08 TR59, FAdV-02 685, FAdV-08a Stanford, IBDV NC171, IBDV 05SA8, and IBDV Del A.

We found that Ontario broiler flocks were exposed to FAdV, IBDV, and CAV. Potentially pathogenic genotypes of FAdV and IBDV that can guide vaccine development and disease control efforts in Ontario were identified. Broiler districts and seasons to target for further research were also identified.

EPIDEMIOLOGY AND ANIMAL HEALTH ECONOMICS

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The epidemiology of *Brachyspira* species in Ontario layer chicken flocks

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Avian intestinal spirochetosis is a disease of birds caused by the spirochete bacterium *Brachyspira*, and is characterized by increased mortality, reduced egg production, weight loss, diarrhoea, and fecal staining of egg shells, which leads to down grading of eggs. This study was initiated by egg grading companies to address the concerns of the companies and the Canadian Food Inspection Agency in relation to dirty eggs in the Ontario layer industry. Our objectives were to investigate the presence of *Brachyspira* species in Ontario layer chicken flocks with downgrades for dirty eggs (dirty flocks) and flocks without downgrades for dirty eggs (clean flocks); determine if there was an association between *Brachyspira* and egg status (dirty vs. clean flocks); and identify factors associated with the presence of *Brachyspira* species.
Fresh pooled fecal samples were collected from 89 flocks on 58 farms and submitted to the Animal Health Laboratory for testing by RT-PCR. Further, farm interviews were conducted to determine risk factors associated with presence of *Brachyspira* species. Multilevel logistic regression model was used to identify risk factors associated with presence of *Brachyspira* species. *Brachyspira* species were detected from 47.2% of the flocks. The odds of *Brachyspira* species among dirty flocks were higher (OR = 18.3; 95% CI: 1.4 - 238.3; $P = 0.026$) than clean flocks. The pathogenic species *B. pilosicoli* was isolated from 13.5% and 10.8% of *Brachyspira*-positive dirty and clean flocks, respectively. The odds of *Brachyspira* species were higher among flocks from multi-age farms (vs. single-age farms), flocks ≥ 60 weeks of age (vs. ≤ 34 weeks), flocks housed in A-frame cages with manure curtains (vs. stacked cages), and flocks housed in barns ≤ 14 years of age (vs. ≥ 30 years). This study provides valuable information on *Brachyspira* species as a potential cause of fecal-staining of egg shells in Ontario, and factors associated with its presence.

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Post-vaccination monitoring and surveillance for Highly Pathogenic Avian Influenza in Long An Province, Vietnam, 2009: design and findings

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In 2009 Vietnam has conducted post-vaccination monitoring and surveillance for Highly Pathogenic Avian Influenza (HPAI) as part of the HPAI national control program. Long An province, located southern part of Vietnam, is an area with repeated HPAI outbreaks in poultry. This presentation is to describe a study aimed to demonstrate the value of estrablishing an HPAI post-vaccination monitoring and surveillance program in Long An province. Long An province was selected for the implementation of the program as a prototype for similar regions. Stratifed sampling scheme was implented using flock size and avain species for four different strata. Samples were collected from domestic avian species for virological and serological testing using real-time PCR with pooled samples of tracheal or cloacal swabs and Haemagglutination Inhibition Test (HI) of individual serum samples respectively. A poultry flock that has average antibody titer≥70% was considered as immunologically protected and a flock that has at least one positive virological test was considered as infected. Factors such as avian species, vaccination history, age and flock-size were considered in the analysis. The analysis was performed using multivariate logistic regression. The analysis included factors that are significant at a p-value<0.10 obtained from bivariate analysis. A total of 324 (4,840 individual samples) and 318 (1,937 pooled samples) poultry flocks were tested for serological and virological purposes respectively. The serological results showed that 48% of vaccinated flocks were immunologically protected and 6% of poultry flocks were infected per the virological testing. The logistic regression indicated that vaccination status and older age avian (>60 days) are significantly associated with protection status as well as the infection status. Our findings indicated that the HPAI virus is still circulating in Long An province despite the efforts to control the infection. Vaccination is an effective tool to reduce HPAI infection, however, low immunity level among the vaccinated flocks may contribute to mainntaince of the virus in the populations.

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Evaluation of molecular profiling tools to differentiate strains of Salmonella Enteriditis

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Currently, pulsed field gel electrophoresis (PFGE) remains the gold standard method for typing food-borne pathogens, including *Salmonella* Enteriditis. However, *S.* Enteriditis exhibits a particularly high degree of clonality within its population, making it difficult to discriminate using modern typing tools. Here we evaluated PFGE, as well as multi-locus sequence typing (MLST), virulence genotyping, and multi-locus variance analysis (MLVA) for their ability to determine the relatedness of 21 *S.* Enteriditis strains from human, poultry, and environmental sources associated with a peritonitis outbreak in laying hens. Antimicrobial susceptibility analysis using the National Antimicrobial Resistance Monitoring Scheme (NARMS) panel was used to determine antimicrobial susceptibility to 15 antimicrobials. PFGE analysis using two different enzymes (*XBaI* and *BlnI*) and post restriction analysis using BioNumerics software grouped the isolates into 4 and 5 clusters each. All isolates were identified as Sequence Type 11 (ST11), belonging to the ST complex 4 using MLST analysis. Virulence genes were detected in most isolates, indicating wide distribution within the serotype. NARMS analysis found all isolates were susceptible to all antimicrobials tested. Insufficient discrimination of *S.* Enteriditis by PFGE, MLST, virulence genotyping, and antimicrobial susceptibility analysis indicates the need for a more discriminatory typing tool. Results of MLVA show promise as a differentiation tool because it is based on highly variable short repeat sequences that are dispersed throughout the genome. These results will be valuable for food safety agencies when identifying pathogen origins for highly clonal serotypes of *Salmonella*.

EPIDEMIOLOGY AND ANIMAL HEALTH ECONOMICS

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Comparison of PCR assays for reliable, early and fast detection of PRRSV in different sample types from experimentally infected boars

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The aim of this study was to compare the sensitivity and specificity of commercially available PRRSV diagnostic assays to detect genetically diverse isolates of PRRSV in different sample types (serum, semen, blood swabs, oral fluids). Fifteen PRRSV naïve boars were divided into 5 groups of 3 boars and inoculated with one of five PRRSV isolates (VR2385, SDSU73, JA142, FL12 or 2010011381). Samples were collected on days post inoculation (dpi) -2, 1, 3, 5, 7, 14 and 21. PRRSV PCR was performed on extracted samples using the following kits: TaqMan® NA and EU PRRSV Reagents (AB, Applied Biosystems); Tetracore U.S. and Euro PRRSV Master Mix (TC, Tetracore) and the AcuPig® PRRSV real time PCR kit (AD, AnDiaTec). At dpi 1, all kits were able to detect at least one positive sample in each group, with the highest detection rates on dpi 3 and 5 for all tested kits. Among the sample types, serum had the highest detection rate reaching a positive detection rate of 90% (54/60) during the acute phase of infection (dpi 1-7) with 100% of agreement between kits, followed by blood swabs and semen samples. Oral fluids samples presented the lowest detection rate and the highest disagreement between kits, with 55, 41 and 46% of positive detection for AB, TC and AD kits respectively. Considering all five PRRSV strains tested, AB kit had the highest detection rates with 67.2% positive samples (242/360), followed by AD (220/360, 61.1%) and TC (218/360, 60.5%). In summary, the detection rate varied depending on the sample type and virus isolate. Serum and blood swabs had the best overall performance with the highest detection rates and agreement between kits. The AB kit had the highest detection rate across all PRRSV isolates used in this study.

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Swine influenza virus dynamics in sow herds over time

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The objective of this study is to describe the dynamics of virus infection in breeding herds overtime and to evaluate the role of replacement animals and piglets on the introduction and maintenance of influenza A virus (IAV) in sow herds.
Five conveniently selected herds were enrolled for this study and are currently being followed for a period of 12 months starting on November 2011. Only sow farms with the gilt developing unit on site and without commercial growing pigs on site were selected. In each farm three populations are being sampled each month: 3 week-old suckling piglets, gilts that have been on site for more than 4 weeks and gilts that have been on site less than 4 weeks. From each sub-population, 30 nasal swabs and a maximum of 10 oral fluid samples are collected. Phylogenetic analysis will be developed to assess the genetic association within and between viruses found in the same herd.
All farms have tested positive at least once to swine IAV by RT-PCR during the sampled months. Overall pig prevalence will be showed at the meeting.
Swine IAV transmission in endemic infected herds appears to be very dynamic within and between sub-populations (piglets, gilts, and young gilts) found in breeding herds . />Acknowledgments: National Institute of Allergy and Infectious Diseases, National Institutes of Health Centers of Excellence for influenza Research and Surveillance, specially the MCEIRS, Minnesota Super Computing Institute and the BioMedical Genomic Center of the University of Minnesota

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Antimicrobial susceptibilities of *Escherichia coli* isolated from feces of swine fed with chlortetracycline or copper

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Purpose: to characterize tetracycline and ceftiofur susceptibilities of 569 *E. coli* isolated from feces of piglets experimentally fed chlortetracycline and/ or copper.
Methods: five piglets per pen were randomized to receive chlortetracycline (CTC), copper, CTC plus copper or control. We determined the minimum inhibitory concentration (MIC) of *E. coli* against a panel of 15 drugs. Furthermore, PCR targeting *tet*(A) and *tet*(B) encoding for tetracycline resistance and bla_{cmy-2} gene for ceftiofur resistance was also used. Data were analyzed by mixed effects logistic regression, survival analysis, bivariate and multivariate probit regression and McNemar's test in STATA 12.
Results: Both survival analysis and mixed effects logistic regression showed day 7 isolates had a significantly increased resistance to ceftiofur. However, this resistance waned overtime. Mixed effects logistic regression showed that chlortetracycline supplementation significantly increased tetracycline resistance as expected. Both mixed effects logistic regression and probit regression showed that copper by CTC interaction significantly increased the probability of *tet*(A) detection while significantly reducing that of *tet*(B) ($P<0.05$). The prevalence of *tet*(A) and bla_{cmy-2} showed age dependent reductions as the piglets got older. There was a significant and strong positive association between *tet*(A) and bla_{cmy-2} genes. However, there was a significantly strong, but negative, association between *tet*(A) and *tet*(B) and between *tet*(B) and bla_{cmy-2}. A significantly higher proportion of *E. coli* isolates with MIC values of ≥ 32 µg/ml for tetracycline had *tet*(B) versus *tet*(A) ($P<0.0001$). *tet*A and bla_{cmy-2} genes were significantly associated with higher multidrug resistance (MDR) while *tet*(B) was significantly associated with lower MDR ($P<0.001$).
Conclusions: In conclusion, there was an age dependent reduction of ceftiofur and tetracycline resistances and the synergy between copper and CTC has a differential effect on *tet*(A) versus *tet*(B) detection.

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Role of environment in the persistence of antimicrobial resistant Salmonella in antimicrobial free (ABF) and conventional pigs at farm and slaughter

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Purpose: The aim of this longitudinal study was to determine the prevalence and molecular characterization of multidrug resistant (MDR) Salmonella in ABF and conventional pigs at farm ,slaughter and in their environment.

Methods: Samples from a cohort of 35 pigs per farm and their environment (feed, water, soil, lagoon, truck and floor swabs) were collected at 10 conventional and 8 ABF farms at different stages on farm (farrowing, nursery, finishing) and slaughter (post-evisceration, post-chill, mesenteric lymph nodes). A total of 2889 fecal and 2122 environmental samples were collected at farm. In addition, 1363 slaughter and 205 lairage and truck samples each were collected at slaughter. Salmonella was characterized for their antimicrobial resistance profile, resistance genes and class I integrons. Genotypic relationships among Salmonella isolates were determined by Pulsed-field gel electrophoresis (PFGE).

Results: A total of 1090 Salmonella were isolated. Salmonella prevalence on conventional farms was significantly higher in pigs (4%; n=66) and environment (11.7%; n=156) compared to the ABF pigs (0.16%; n=2) and environment (0.62%; n=5) ($P < 0.01$). At slaughter, Salmonella was isolated from all the stages including post chill. There was no statistically significant difference between ABF and conventional carcasses prevalence (P=0.94). The isolates exhibited highest frequency of resistance to tetracycline including from conventional farm environment (88%) and pigs (82%) followed by ABF pigs (60%) and their environment (21%). MDR (resistance to ≥ 3 antimicrobials) was detected in 23% (n=257) of the isolates. Salmonella isolates from pigs and environmental samples at farm and slaughter exhibited similar resistance and fingerprinting profiles by PFGE. We detected blaTEM, blaPSE, cmlA, str(A), (B), aad (A1), (A2), tet A and blaCMY-2 resistance genes by PCR. The MDR isolates carried class 1 integrons of 1.0 and 1.2kb size.

Conclusions: The phenotypic and genotypic results of our study indicate the role of environment in the transmission of AR Salmonella in the two production systems. The prevalence of AR Salmonella in ABF pigs in the absence of selection pressure is concerning.

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Risk factors for environmental contamination with *Salmonella enterica* in a veterinary teaching hospital

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Salmonella enterica is commonly recognized as a cause of nosocomial infections as well as zoonotic infections in veterinary teaching hospitals (VTHs). The objective of this study was to determine risk factors associated with environmental contamination of a veterinary teaching hospital with *S. enterica*.

Environmental surveillance samples were collected from February 2003 through June 2011, using a commercially available electrostatic wipe, as part of the ongoing infection control program. Sampling sites included both floor and hand contact surfaces throughout the VTH. Risk factors evaluated included hospital case load, hospital use areas, severity of disease, presence of culture positive inpatients and season. Data on risk factors of interest were collected retrospectively from the VTH medical records database. Multivariable logistic regression was used to evaluate associations between hospital risk factors and veterinary hospital environmental contamination with *S. enterica*.

During the study period, approximately 53 samples were collected monthly, for a total of 5337 environmental samples. Of the samples collected, a total of 7.9% (n=423) were culture positive for *S. enterica* using standard culture techniques. In general, environmental samples collected in the Food Animal Hospital and floor samples were more likely to be positive.

Risk factors identified in this study will allow for the refinement of existing infection control programs as well as provide guidance to those in program development. A better understanding of the risk factors associated with environmental contamination will allow for more practical evidence based preventive measures to be implemented in veterinary hospitals experiencing epidemics of nosocomial infections with *S. enterica*.

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Perceptions of veterinarians and producers concerning Johne's disease in US beef cow-calf operations

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Purpose: The purpose of this study was to compare the perceptions of producers and veterinarians on the burden of *Mycobacterium avium* subspecies *paratuberculosis* infection in cow-calf herds and activities to control new infections.

Methods: Mailed questionnaires were sent to beef producers through the Designated Johne's Coordinators and to veterinarians using the database of a nationwide professional organization.

Results:Twenty-two percent (34/155) of producers reported having infected animals in their herds. Seedstock herds ($P = 0.039$) and herds currently enrolled in a control program ($P = 0.024$) were more likely to report being uninfected than other herd types. Average (minimum, median, maximum) animal-level prevalence reported by producers was 0.8% (0, 0, 10). Average producer-estimated prevalence increased with increasing herd size. A total of 27 of 100 respondent producers reported having at least one clinical animal during the previous year. Seedstock producers were more enthusiastic about Johne's disease (JD) control programs and had a correspondingly lower prevalence. Average veterinarian-estimated animal- and herd-level prevalence were 5% (0, 2, 60) and 27% (0, 10, 100), respectively, in client herds. Average veterinarian-estimated within-herd prevalence was 9% (0, 5, 60) for infected herds. Producers reported implementing measures to control MAP transmission more frequently than perceived by veterinarians, but few differences were statistically significant. Most herds did not implement control activities expected to aid in control of MAP transmission including testing herd additions, early weaning, and strategic management of calves from suspected dams. Testing recommendations by veterinarians for beef cow-calf herds were bacterial culture of feces (n=10), PCR (n=39), ELISA (n=97) and a combination of these tests (n=131). The recommended interval between testing was 12 months by 79% (198/252) respondent veterinarians.

Conclusions: Describing discrepancies between veterinarian and producer perceptions is important to identify educational activities that may improve management and control of Johne's disease.

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Effect of individual animal calving pens on peripartum transmission of Mycobacterium avium subsp. paratuberculosis in Holstein heifer calves.

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Purpose: Individual animal calving pens that are cleaned between successive calvings is recommended as a strategy for blocking MAP transmission in calves during the preweaning period. In this study, we quantified the efficacy of individual-animal pens cleaned between uses (versus multiple animals calving pen) for preventing periparturient transmission of MAP in Holstein calves.
Methods: Every other pregnant cow or heifer in 3 MAP endemic Minnesota herds was moved to either the individual-animal calving pens (treatment group, n =238) or the multiple animal calving areas (control, n = 211) within 48 h to 72 h prior to expected calving. Each calf born in the individual-animal calving pen was assigned to the treatment group; or control group if born in the multiple animals calving pens. Calves were separated from their dams shortly following birth. The study intervention used within individual-animal calving pens was a higher level of hygiene relative to that in the multiple animal calving pen, achieved by removing fecal material in the individual animal calving pen immediately after each birth. Calves were monitored into adulthood and then tested at approximately 24, 48, and 60 months of age using a commercial serum ELISA and bacterial fecal culture for MAP. Cox regression models were used to quantify the hazard of MAP infection in heifers 24 months and older born in multiple animal calving pens relative to the hazard in herd mates born in individual animal calving pens after adjusting for MAP infection status of the dams.
Results: Hazard of MAP infection based on positive bacterial culture test outcomes was just over 3-fold higher in cows born in multiple (vs. individual) animal calving pens (HR = 3.362, 95% CI = 2.371 to 4.766). Considering the ELISA test outcomes, the hazard of MAP infection was approximately 0.75 times higher in cows born in multiple (vs. individual) animals calving pens (HR = 1.752, 95% CI = 1.158 to 2.651).
Conclusion: Use of multiple animals pens for calving increases the likelihood of MAP infection in dairy cattle. Utilizing individual animal pens with feces removed between births for calvings is an effective strategy for reducing MAP transmission risks in calves.

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Effect of delayed exposure of dairy cattle to Mycobacterium avium subsp. paratuberculosis on age at first test positive and clinical Johne's disease

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This is an observational study that evaluated the effect of delaying exposure of cattle to Mycobacterium avium sp. paratuberculosis (MAP) on the incidence of MAP test positivity using bacterial culture of feces and serum ELISA and clinical Johne's disease (JD) during the subsequent three years of life in dairy herds.
Two contemporary birth cohorts of dairy cows were followed through 3 years. An unexposed birth cohort (UBC) of 79 dairy cows was matched with an exposed birth cohort (EBC) of 260 dairy cows. Cows in the UBC were born and raised in 5 MAP uninfected herds and introduced into the 5 MAP infected herds where cows of the EBC were born and raised. Fecal and serum samples were collected from each cow in the study each year and tested using bacterial culture of feces and serum ELISA for detection of MAP. Dates and culling reasons were also collected from herd owners. During the study, we compared the incidence of MAP bacterial culture and serum ELISA test positivity and clinical JD using a time-dependent Cox regression with left truncation and right censoring.
The hazard of positive bacterial culture, positive serum ELISA and clinical JD in cows in the UBC was 0.12 (0.06-0.23), 0.03 (<0.01-0.19) and 0.001 (<0.001-0.3) times lower compared to cows in the EBC, respectively; however, this difference decreased 2.2% (0.1 to 4.3), 6.2% (3.3 to 9.2) and 17.4% (7.9 27.6) per month of age, respectively.
Delaying the introduction of cattle into MAP infected herds was associated with a lower incidence of test positivity and clinical JD; however this difference decreased as cows became older.

061

Does colostrum intake affect the development of the rectal microbiota in pre-weaning dairy calves?

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The objective of this study was to explore the composition of the dominant rectal bacterial microbiota in pre-weaning dairy calves conditional on age, and total serum protein (TSP) concentrations.
We sampled calves from four farms in Washington state. Fecal samples were collected and pooled within farm by age (1, 2, 4 week old), and TSP status (≥5.2 and ≤5.0 g/dl). Bacterial DNA sequences were obtained by transformation and cloning of ribosomal 16S gene into E.coli DNA libraries. 16S DNA sequences were used for taxonomic identification and investigation of microbiota composition.
A total of 164 calves were enrolled in the study to create 20 pools, and 1824 16S ribosomal DNA sequences were assessed. Using phyla as the first level of analysis, across all pools the calf rectal community was similar to that previously reported in calves, with the two major phyla being Bacteroidetes (52.2%) and Firmicutes (32.1%). The remaining sequences were split between additional phyla (Fusobacteria, Proteobacteria, Actinobacteria, Lentisphaerae and Spirochaetes). At a more specific taxonomic level we detected 59 taxa corresponding mainly to genus level. Major compositional changes were noticed across ages, particularly with *Bacteroides* and *Faecalibacterium* decreasing and *Prevotella* increasing with age. Changes in the relative proportions of the most abundant taxa were also found as *Bacteroides* and *Prevotella* decreased and *Faecalibacterium* and *Fusobacterium* increased in low TSP calves compared to adequate TSP calves. Rarefaction analysis indicated a trend for greater microbial richness in calves with ≥5.2 g/dl compared to ≤5.0 g/dl TSP levels and with increasing age. Clustering analysis demonstrated bacterial grouping by age and TSP.
This is, to our knowledge, the first population based investigation on fecal microbiota in dairy calves during their first month of life, and with adequate and inadequate colostrum intake. Our molecular approach shows new and detailed information on gut microbiota composition and successional changes during the first month of life. Rarefaction and clustering analyses indicated colostrum and age might influence microbial composition in pre-weaned calves.

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Metagenomic versus microbiological culture based approaches to evaluate the effects of interventions strategies on ceftiofur and tetracycline resistance in cattle feces

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Purpose: To determine the effects of 2 intervention strategies (i.e., feeding chlortetracycline (CTC) following ceftiofur (Excede®) treatment and mixing of ceftiofur-treated with untreated animals) on ceftiofur and tetracycline resistances, both at phenotypic and genotypic levels. Methods: A controlled field trial was conducted on 176 steers. Steers were randomly allocated to 16 pens of 11 steers each. The 2 intervention strategies were assigned randomly to the pens in a 2-way full-factorial manner. Fecal samples were collected every other day to 26 days. The bla_{CMY-2}, *tet*A, *tet*B, and 16srRNA gene copies/µl of DNA were determined using qRT-PCR from fecal community DNA. bla_{CTX-M} gene quantification is currently in progress. Antimicrobial susceptibility testing was also performed on 1050 *E. coli* isolates using the NARMS gram negative panel. The relationship of bla_{CMY-2}, *tet*A, and *tet*B genes copies with explanatory variables (CTC and mixing (MIX) in a full factorial design interacting with period (DAY)) were assessed, using separate multi-level mixed models. In addition, a logistic model for discrete-time survival was used to determine the effect of intervention strategies on the phenotypic resistance levels of the isolates towards specific antimicrobials. Results: CTC had a strong increasing effect on bla_{CMY-2}, *tet*A, and *tet*B gene copies consistently across other factors ($P<0.05$). Mixing had a period-specific effect of decreasing the bla_{CMY-2} gene copies inconsistently across other factors. Fitted survival curves indicated that administration of both CTC and ceftiofur selected for isolates with better survivorship at higher *in vitro* ceftiofur concentrations. Conclusions: CTC favors expansion of bla_{CMY-2}, *tet*A, *tet*B gene copies as well as the phenotypic expression of ceftiofur resistance. Mixing has a significant, though inconsistent, sparing effect on bla_{CMY-2} gene copies. A strong positive correlation was seen between bla_{CMY-2} and *tet*A genes ($P<0.05$). Metagenomic results (bla_{CMY-2}, bla_{CTX-M}, *tet*A, and *tet*B gene copies/µl of DNA) will be presented and compared with the concurrent phenotypic analysis of *E. coli* isolates.

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Asymptomatic endemic *Chlamydia pecorum* infections reduce growth rates in calves by up to 48 percent

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Intracellular *Chlamydia* (*C.*) bacteria cause in cattle some acute but rare diseases such as abortion, sporadic bovine encephalomyelitis, kerato-conjunctivitis, pneumonia, enteritis and polyarthritis. More frequent, essentially ubiquitous worldwide, are low-level, asymptomatic chlamydial infections in cattle. We investigated the impact of these naturally acquired infections in a cohort of 51 female Holstein and Jersey calves from birth to 15 weeks of age. In biweekly sampling, we measured blood/plasma markers of health and infection and analyzed their association with clinical appearance and growth in dependence of chlamydial infection intensity as determined by mucosal chlamydial burden or contemporaneous anti-chlamydial plasma IgM. *Chlamydia* 23S rRNA gene PCR and *ompA* genotyping identified only *C. pecorum* (strains 1710S, Maeda, and novel strain Smith3v8) in conjunctival and vaginal swabs. All calves acquired the infection but remained clinically asymptomatic. High chlamydial infection associated with reduction of body weight gains by up to 48% and increased conjunctival reddening ($P<0.0001$). Simultaneously decreased plasma albumin and increased globulin ($P<0.0001$) suggested liver injury by inflammatory mediators as mechanisms for the growth inhibition. This was confirmed by the reduction of plasma insulin like growth factor-1 at high chlamydial infection intensity ($P<0.0001$). High anti-*C. pecorum* IgM associated eight weeks later with 66% increased growth ($P=0.027$), indicating a potential for immune protection from *C. pecorum*-mediated growth depression. The worldwide prevalence of chlamydiae in livestock and their high susceptibility to common feed-additive antibiotics suggests the possibility that suppression of chlamydial infections may be a major contributor to the growth promoting effect of feed-additive antibiotics.

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Livestock Deaths in Mangarabombang Subdistrict, Takalar District, South Sulawesi Province, Indonesia, 2011-2012: Application of Epidemiological Investigation

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An outbreak of deaths with high livestock mortality was reported in two villages in Mangarabombang Subdistrict of Indonesia in May 2012 when an investigation was initiated. The objectives of the presented study were to describe the epidemiological characteristics of this outbreak and to provide plausible recommendations in order to improve the control measures. Conventional steps of an outbreak investigation were followed. A questionnaire was developed to address factors assosiated with the source and spread of the disease. Specimens from suspected animal, soil, bone, and bone meal samples were collected and submitted to laboratory for confirmation of the causative agent. The outbreak started in November 2011, however no official report was made until its peak in May 2012. There were 182 livestock deaths, the majority were cattle (90%) with mortality rate of 31% among all livestock species in suspected households in Laikang and Punaga villages. Three most common clinical signs were reported including trembling (60%), sudden death (54%), and frothy mouth exudates (52%). Most of suspected households reported sharing pasture area (86%) for grazing their cattle. The interviewed farmers did not properly dispose and left their animals carcasses in the field (33%), and 43% of these farmers sold their sick animals to traders for slaughtering. Two samples from dead animals and one soil sample were tested and were confirmed positive for Bacillus anthracis using culture and identification method. A human case was identified by coincidence that has eschar lesion on his thigh 3 days after participating in slaughtering a sick cattle. Penicilline was given to animals in the households with reported animal deaths. This outbreak indicated the importances of farmer's practices in sharing grazing area, left the carcasses in the field and selling sick animal as possible paths of spreading the disease. Contact with animal carcasses while slaughtering was the cause of human infection as report elsewher. Proper animal vaccination program in conjunction with education of farmers about the anthrax are suitable measures to prevent and control the disease.

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Reported livestock diseases in myanmar during 1998-2011: assessment and trend of investigated outbreaks

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The epidemiology of the disease is essential component in designing and implementing an effective disease control program, particularly for countries where resources are limited. In Myanmar, foot and mouth disease (FMD), hemorrhagic septicemia (HS), black leg and anthrax are identified as diseases of economic importance for food production as well as public health concern. Despite availability of data collected during outbreaks through paper records, the overall occurrences and impacts of these diseases have not been described. The objective of this abstract is to present a study conducted to describe selected epidemiological characteristics of the aforementioned diseases in Myanmar using available official outbreak records from 1998 to 2011. Official outbreak records were retrieved from the Myanmar's Livestock Breeding and Veterinary Department (LBVD), including data on locations, date of the outbreak, number of involved livestock species, tentative diagnosis, and laboratory outcome for confirmed cases. Ranking of the importance of the reported diseases was performed using specific epidemiological indices. An indication of the importance of a disease to the total reported sick animals in the outbreaks was measured by proportional morbidity rate. Using this index, cattle / buffalo had the highest for all above mentioned diseases but the magnitude was different among these diseases. FMD had the highest in magnitude of morbidity with the lowest for the anthrax. An indication of the contribution of a disease to the total deaths in these outbreaks was measured by proportional mortality rate. Using this index, cattle / buffalo had the highest for all above mentioned diseases but the magnitude was different among these diseases. The highest contribution to mortality was HS and lowest for FMD. Analyses were based on official records where underreporting is highly plausible. Nevertheless, this was the first study providing a comprehensive review of the existing records of priority animal diseases in Myanmar. The information from this study could be utilized for strategic planning of disease control programs such as vaccination campaign.

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Using quarterly earnings to assess return to function in Thoroughbred racehorses after either modified laryngoplasty or colic surgery

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Purpose: To validate and use quarterly earnings to assess racing performance of Thoroughbreds (TB) after either modified laryngoplasty (ML) for treatment of recurrent laryngeal neuropathy or abdominal surgery (CS) for acute colic. Design: Retrospective cohort. Animals: TB racehorses after ML (N=70) and untreated cohorts equivalent in class (N=210). TB racehorses 2-5years old that had CS and survived to hospital discharge (N=59) and their untreated cohort (N=90).
Methods: Medical records(treated horses) and race records (all horses) were reviewed. Summary statistics generated for each horse were total races, race winnings, quarters(Q) racing, average races per Q, earning Q, dollar winnings per earning Q, and Q idle (i.e. Q without races where records showed subsequent races). Total racing career = Q racing + Q idle. Summary comparisons were by means of Wilcoxon's rank sum (continuous) or Kruskall Wallis (ordinal variables) tests. Racing longevity was assessed using survival analysis. Prior to comparing study groups, comparisons were made between subgroups of untreated cohorts to ensure that randomly selected groups of untreated horses were not different.
Results: There were no differences between untreated subgroups. In the last pre-surgery race, ML horses performed significantly ($P < 0.001$) worse than untreated horses, whereas CS horses performed as well as their peers. All ML horses had at least one race post-surgery but only 76% of CS returned to racing. Median Q of follow-up racing data for ML and CS horses and their cohorts were 8 and 32, respectively. With the exception of Q1 post-surgery for horses undergoing ML and Q1-3 for those after CS, there were only slight differences in race starts and no significant differences in earnings between treated and untreated cohorts. For horses that raced after surgery, there were no differences in cumulative survival compared to untreated horses.
Conclusions: Quarterly earnings can provide a more detailed longitudinal assessment of a racehorse's performance. Horses treated by ML or CS return to similar levels of performance as their untreated cohorts by Q2 and Q4 after surgery, respectively, and continue to compete as long as their cohorts.

067

Minimization of bovine tuberculosis control costs in US cattle herds

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Purpose: The objective of this study was to minimize the cost of controlling an isolated bovine tuberculosis (bTB) outbreak in a US dairy herd.
Methods: A stochastic simulation model of bTB was used to predict the biologic and demographic outcomes of test-and-removal programs with testing intervals (TI) of 2 to 12 months and between 2 and 8 negative whole herd tests (NWHT) required for the herd to be declared clear. Test- and removal costs were tabulated on a monthly basis. Farm-level costs included value foregone and cost of replacement less indemnity for all cows culled by testing plus holding costs due to quarantine. Government-level costs included testing costs plus indemnity less salvage for all cows culled by testing. Depopulation costs were calculated at the time of detection for all herds. Farm costs for indemnity were the sum of the cost of replacement less indemnity for all cows, holding costs, and the net milk profit lost, assuming a 3-month holding period before repopulation. Government costs were indemnity plus post-mortem testing less salvage value for all cows. The model was optimized separately with regards to cost for farm and government, using TI and NWHT as control variables and a non-parametric optimization algorithm run for 1 million iterations. All costs were parameterized for an average dairy herd in California. Results: First-order dominance was observed for both cost levels at NWHT=2. The optimizer showed a slight, non-significant preference for longer testing intervals at both cost levels, but did not clearly prefer any TI between 3 and 12 months. If farm-level holding costs were added, however, the farm-level optimizer significantly preferred a 2-month testing interval. In all cases, the distribution of test-and-removal costs was lower than and did not overlap the distribution of depopulation costs, although the distributions were less distinct for farms with high holding costs.

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Conclusions: Based on the results of this model, we recommend annual testing of herds after an outbreak of bovine tuberculosis, with 2 negative whole herd tests being sufficient to lift quarantine. If costs are associated with the length of quarantine, shorter testing intervals could be preferred.

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Patterns of cattle farm visitation by white-tailed deer in relation to bovine tuberculosis transmission risk in Minnesota
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The objective of this study was to characterize spatial patterns of white-tailed deer movement related to bovine tuberculosis (BTb) transmission risk to cattle in northwestern Minnesota. Sixteen adult deer (12 females and 4 males) were captured in January 2011 and fitted with GPS collars just outside the BTb Management Zone in a 140 mi2 study site that represented transitional deer habitat interspersed with cattle operations (n ≈30). This area was similar in habitat composition to the Minnesota BTb Core area, in that free-ranging deer could move east into the forest zone, west into the farmland zone, or remain in the transition zone. GPS collars were programmed to collect location information every 90 minutes. Ground-truthing was performed seasonally to assess the locations of fenced cattle, cattle feeding areas, and feed storage areas potentially visited by deer. Due to unexpected high winter mortality (38% between January and March 2011), mostly due to wolf predation (83%), a second capture was performed in March 2011 which added 5 deer (4 females and 1 male) to the study. Ten deer (48%) remained in the study until the end date by April 2012. Results show that five deer (25% of total deer) had locations within farms and these occurred on six cattle farms (20% of total farms). Most of the visits occured in areas where cattle was present (77%). Two deer visited only one farm, two deer visited two farms and one visited three farms. The Spring months, from March to May, had the most deer farm visitations (37%). A higher proportion of visits occurred from 12 am to 6 am (60%). These study results provide baseline information regarding cattle-deer interactions critical to transmission of BTb in this region.

069
A comparison of real and synthetic population datasets for simulation modeling of highly pathogenic avian influenza (H5N1) in commercial poultry flocks in South Carolina.
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Purpose: Spatially explicit models of the spread and control of disease in populations of livestock and poultry rely heavily upon valid spatial representations of the populations of interest, including such characteristics as the geographic locations of farms and their proximity to other susceptible farms in the population. In the United States, limited information is available regarding the locations of actual farm premises; therefore, modeling work often makes use of artificially generated (i.e., synthetic) population datasets. In order to better understand the potential effects that a population dataset may have on model outcomes, and to evaluate the accuracy and validity of the use of such synthetic datasets, we compared the outcomes of mechanistic epidemiologic simulation models that were run using an accurate population dataset to those of models that made use of several different synthetic population datasets.
Methods: We simulated the spread and control of the H5N1 strain of highly pathogenic influenza among commercial poultry in the state of South Carolina, a system that was recently well characterized for the purposes of epidemiologic simulation modeling.
Results: We found that there was generally good qualitative agreement among models run using various population datasets, but that the quantitative differences in model outcomes could be substantial.
Conclusions: When quantitative outcomes from epidemiologic models are desired or required, care should be taken to adequately capture or describe the uncertainty in model-based outcomes due to the use of synthetic population datasets.

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Density and distribution of backyard poultry flocks in metropolitan Denver, Colorado
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Backyard poultry flocks are not well characterized, and prevalence of these flocks in the U.S. is unknown. Backyard flocks have been involved in economically significant nationwide avian disease outbreaks and flock owners are considered at risk for infection with zoonotic poultry diseases. This study was conducted to determine backyard poultry flock prevalence and distribution, understand flock characteristics and human-bird interactions, and to better understand the potential for introduction of foreign animal and avian zoonotic diseases in a typical urban-suburban area. A cross-sectional survey was conducted of census block groups in urban and suburban areas of Denver, Colorado. Flock distribution data were compared to U.S. Census demographic data to determine potential predictive characteristics for poultry ownership.
Poultry flock prevalence in the Denver metropolitan area was 2.2% overall (1.55% urban, 4.40% suburban). Egg laying chickens were the most common birds raised (79%). The most common reason for owning birds was food production for the family (50.7%). Bird movements were rare (13% of flocks); however, all bird owners travelled to places where other birds were located. Flocks raised for show purposes were more likely to be large flocks (p=0.001), were more likely to travel (p=0.007), and were the only flocks that traveled out of state. Demographic variables related to poultry ownership included married childless couples, single females, and housing values above $200,000. Poultry were more likely to be found in census block groups having larger lot sizes and lower population per square mile.
Our study identifies potential mechanisms for avian disease introduction to backyard flocks, and describes characteristics of poultry ownership in

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a typical urban-suburban area. This information may be useful for targeted surveillance and public education efforts in the event of an avian foreign animal or zoonotic disease outbreak.

071
Antimicrobial resistance in fecal E.coli of Holstein calves housed individually or in group pens.
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Abstract Not Available

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Herd prevalence and geographic distribution of *Coxiella burnetii* in cattle bulk tank milk samples in Indiana
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Objective: To determine the herd prevalence and geographic distribution of *Coxiella burnetii*, the causative agent of the zoonosis Q fever, in milk samples from commercial cattle dairies in the state of Indiana, United States.
Methods: A total of 1385 bulk tank milk samples representing over 95% of commercial cattle dairies in the state were provided by the Indiana State Board of Animal Health. These samples were identified by county and region (Northern, Central or Southern). A minimum subset of 300 samples was selected for evaluation based on probability proportional to size. In counties represented by only a single sample, that sample was tested. Real time PCR targeting the IS1111 segment of the for the IS1111 segment of the *C. burnetii* genome was performed on extracted DNA.
Results: Greater than 50% of the samples tested show evidence of the presence of the *C. burnetii* IS1111 gene. Positive samples are distributed throughout the state of Indiana.
Conclusion: *Coxiella burnetii*, the bacterium responsible for Q fever is present in milk from dairy cattle in Indiana. Bulk tank samples by definition include milk from multiple cows. In this study, the mean herd size was 96 cows with herd sizes ranging from 2 to 2150 animals. This limits the determination of individual prevalence of shedding of *C. burnetii* among dairy cattle in Indiana. Nevertheless, the presence of *C. burnetii* in this population indicates the possibility of transmission of Q fever from cattle to humans in a commercial dairy setting in Indiana. These results also offer continued support for pasteurization of dairy products to control human exposure to *C. burnetii* through ingestion of products containing the bacterium.

073
Prevalence, distribution, and diversity of Salmonella subtypes on Michigan dairy farms in 2000-2001 and 2009.
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Purpose: Dairy farms are an important reservoir for *Salmonella* infections in humans. Cross-sectional data from the National Animal Health Monitoring System in the U.S. suggest the prevalence of *Salmonella* on dairy farms is increasing. The objective of the following research is to determine overall-and within farm changes in the prevalence and distribution of *Salmonella* subtypes over time. The hypothesis tested by this research is that the prevalence, distribution, and diversity of *Salmonella* subtypes on Michigan Dairy farms is different between 2000-2001 and 2009.
Methods: Retrospective data and stored *Salmonella* isolates from a 2000-2001 study on Michigan dairy farms were retrieved. In 2009, fecal and environmental samples were collected from the same farms using comparable sampling techniques. For farms positive in both time frames, *Salmonella* subtypes were identified using multi-locus sequence typing and pulsed field gel electrophoresis. A generalized linear mixed model was used to test differences in the overall prevalence between years, and a chi-square test was applied to determine differences in the distribution of subtypes between time frames. Differences in simpson's index of diversity were determined by calculating confidence intervals using a normal approximation.
Results: The prevalence of *Salmonella* was significantly higher in 2009 relative to 2000-2001. This coincided with a significant shift in the distribution between time frames towards subtypes belonging to the C1 serogroup. Despite the differing distributions, there was substantial overlap in the population of subtypes: 80% of the isolates recovered in 2009 were STs that were previously recovered in 2000-2001, and 12% of the isolates in 2009 were pulsotypes that had previously been recovered. The diversity of *Salmonella* pulsotypes was significantly higher in 2009 relative to 2000-2001.
Conclusions: These data suggest that the population of *Salmonella* in 2009 was distinct, more prevalent, and more diverse relative to the population of *Salmonella* from the same farms in 2000-2001.

074
Salmonella enterica in lymph nodes of cull and fed cattle at harvest
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The objective of this study was to evaluate the potential association between Salmonella enterica burden within bovine subiliac lymph nodes (LNs) and animal type, season, and region of harvest. Bovine LNs (n = 1,712) were collected from 12 abattoirs, 8 that primarily harvest fed cattle, 2 that primarily harvest cull or dairy cattle and 2 that harvest both fed and cull. Plants were located in BIFSCo regions 2, 3, 4, 5, and 8 (n= 3, 5, 2,

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1, 1, respectively). Subiliac LNs were collected during the months of February through June. LNs were trimmed of surrounding fat, surface sterilized by submersion in boiling water for 3 seconds, pulverized in individual bags with a rubber mallet, and homogenized for 2 minutes at 230 revolutions per minute. Samples were then enriched in tryptic soy broth for 12h at 42C and immunomagnetic separation was performed on the enrichments using paramagnetic beads. Beads were transferred to Rappaport-Vasiliadis broth for secondary enrichment at 42C for 18-20h, and then plated onto xylose lysine desoxycholate and brilliant green sulfa agars and incubated for 18-20hrs at 37C. Morphologically characteristic isolates were serotyped and their susceptibility to a panel of 15 antimicrobials determined. Observed median percent prevalence estimates were 1.3, 2.7, 0.0, 1.3 and 0.0, for regions 2, 3, 4, 5, and 8, respectively. Overall, mean prevalence in feedlot cattle was 4.6% while that for cull cattle was 2.1%. Salmonella was detected in 66 (3.8%) LNs and 35 had quantifiable levels of Salmonella that varied from <1.6 to 4.6 log10 cfu/LN. Preliminary serotyping data (n = 38 isolates) indicate the most common serotypes recovered were Anatum (31.6%), Mbandaka (15.8%), and Montevideo (13.2%). To date, antimicrobial susceptibility testing has been performed on 26 of the 66 isolates. The majority (69%) of the isolates were pansusceptible; however, the ACSSuT resistance phenotype was observed in 19% of the isolates. Our preliminary data confirms the relatively rare occurrence of Salmonella in LNs of cattle during the winter and spring period. Collection of LNs will continue through winter 2013.

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Salmonella recovery from the peripheral lymph nodes following intradermal administration and evaluation of a commercially-available *Salmonella* vaccine

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Research on the prevalence of *Salmonella* in peripheral lymph nodes (PLN) of cattle suggests that regional and seasonal differences occur with the seasonal fluctuations a reflection of fly populations. However, it is not known if *Salmonella* positive PLN arise from transdermal *Salmonella* inoculation. Therefore, three pilot studies were conducted to develop a cattle model in which mimicked fly bites could be examined for their potential to transmit *Salmonella* to the PLN. In the first study, Holstein steers were administered *Salmonella* via needle and syringe, intra-dermally above the metacarpus and metatarsus. *Salmonella* positive PLN were cultured from the inoculated animals while the control steers were all *Salmonella* negative. Swelling and lameness in the treated steers indicated some of the *Salmonella* was delivered subcutaneously, therefore the second and third pilot studies utilized an allergy skin testing device for *Salmonella* inoculation. Steers were administered the *Salmonella* in the lower legs as above and necropsied up to 8 days later. Superficial cervical and popliteal lymph nodes were *Salmonella* positive in both studies and no swelling or lameness was observed in any of the treated steers. The final experiment examined the ability of a commercially-available SRP *Salmonella* vaccine to prevent *Salmonella* positive PLN following intradermal inoculation. Sixteen steers were allotted to control or vaccine treatments and within each treatment, inoculated intra-dermally with either *Salmonella* Montevideo or Newport (lower legs) and Senftenberg (paunch region, all steers) 14 days following the administration of the vaccine booster. Three and six days following *Salmonella* inoculation, steers were necropsied and PLN cultured. The vaccine treatment decreased ($P < 0.05$) the percentage of *Salmonella* positive left superficial cervical and right sub-iliac lymph nodes compared to control steers. Results herein, demonstrate the transdermal uptake and transmission of *Salmonella* to the PLN in cattle and present an animal model for the evaluation of potential pre-harvest interventions to prevent and/or eliminate lymph node acquisition of *Salmonella*.

076

Sub-optimal thermal environment is associated with Salmonella shedding in swine.

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The objective of this study was to evaluate the association between the thermal environment in the barn and Salmonella status in finishing pigs. Individual fecal samples from 900 finishing pigs (8 collections per pig) were planned for collection from 18 cohorts (50 pigs per cohort) in 3 sites of a multi-site farrow-to-finish production system in a longitudinal study. Pen temperature and humidity were measured every 2 minutes during the study period. The thermal parameters of interest were: hourly average, highest, and lowest temperatures as well as hourly temperature variation, for 6 time periods prior to the time of fecal sampling (12h, 24h, 48h, 72h, 1 week and 1 month). Additional potential risk factors at the individual, cohort and pen levels were evaluated. Multilevel logistic models with random intercepts at pig, pen and cohort levels to account for clustering were constructed. The outcome variable was Salmonella status of the individual sample. Salmonella was isolated from 453 (6.6%) of 6836 individual fecal samples. Exposures to temperatures below and above the pig thermal of neutral zone health, nursery Salmonella status, mortality, site and age were associated with Salmonella shedding. Control of the thermal environment in the barn may contribute to reduce Salmonella in swine and will improve animal health and production.

077

A mathematical model to quantify effectiveness of cleaning as a measure to control Salmonella Typhimurium on a grower-finisher pig farm

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Salmonella Typhimurium (STM) is a major foodborne pathogen of public health concern. Infected pigs are the main source of infection for humans, as the bacteria shed with their feces contaminate the environment and the pork products during slaughter. Cleaning of barn surfaces has always been used for on-farm infection control of STM in grower-finisher pig herds. However, the effectiveness of cleaning in the control of STM infection has never been quantified. The aim of this study was to quantify the efficiency of cleaning on controlling STM transmission in a grower-finisher pig herd managed under all-in/all-out production system. To achieve this objective we developed a mathematical model of STM transmission to assess the effectiveness of different levels of cleaning (pathogen removal) for reducing STM prevalence in grower-finisher pig herds. The model predicted that increased cleaning reduces the STM transmission, but this measure alone is unlikely to be sufficient to reduce the infection transmission to levels low enough to prevent an outbreak. That is because the STM transmission is greatly influenced by the bacterial shedding level in the feces of infected pigs. Without some additional measure to lower the level of bacterial shedding in feces, even a highly effective cleaning and sanitation program (e.g., removal of 99% of the pathogen in the environment by cleaning once a day) will not be sufficient

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to prevent an outbreak of STM. A better control program would be to include some measure of reducing bacterial load in feces of infected pigs, such as vaccination, and/or identification and isolation of heavy shedders, with the routine cleaning practice on-farm.

078
False attribution: the effects of bias in probabilistic source attribution models for Salmonella infection
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Purpose: The aim of probabilistic food source attribution models is to quantify the relative contribution of specific foods to the overall burden of human disease. The objective of this study was to assess the accuracy of a Bayesian method of attributing animal-food sources to human salmonellosis cases originally described by Hald et. al (2004).
Methods: The Bayesian model, based on a Monte Carlo Markov Chain (MCMC) was modified to assess 5 Salmonella serotypes and 5 animal-food sources. This model included a measure of consumption of each animal-food source (Mj), the ability of a given food source to act as a vehicle for Salmonella (aj), a measure of serovar pathogenicity (qi), and the prevalence of each serovar in each animal-food source (pij). Scenarios with known expected results were tested in the model, and convergence of the model was assessed with the Gelman-Rubin diagnostic. Model predictive potential was evaluated by comparing prior distributions with posterior frequency histograms for model parameters.
Results: The model was able to attribute the correct number of cases of salmonellosis to each animal-food source when each Salmonella serovar was uniquely associated with one animal-food source. When multiple animal-food sources were contaminated with the same Salmonella serovar, the model exhibited considerable bias. Similarly, when the consumption of the animal-food sources was unequal, attribution deviated significantly from the proportionality assumption. The addition of a serovars-food source interaction term improved model accuracy but further exacerbated problems with model identifiability.
Conclusions: The original model proposed by Hald et al. is over parameterized, and therefore, simplifying assumptions have to be made that lead to inaccurate estimates of attribution. The allowance for an interaction between serovars pathogenicity and food source enhanced the accuracy of attribution but increased the over-parameterization. Given these results, it appears that interventions based on modified version of this attribution model could be misguided.

079
The role of flagella in the attachment of *Salmonella* enterica serovar Kentucky to broiler skin.
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Salmonella Kentucky has been identified as the most commonly isolated serovar in poultry production and processing. To better understand the mechanisms of attachment of *Salmonella* to broiler skin a bioluminescent-based mutant screening was employed. A random mutant library of a field isolated strain of salmonella Kentucky carrying bioluminescent marker (lux CDABE) was constructed. Mutants' attachment to chicken skin was assessed in 96-well plates containing 6mm circular sections of chicken skin and a mutated bacterium. After washing steps, mutants with attenuated attachment properties, according to their bioluminescence, were selected and transposon insertion sites were mapped. Several different mutated genes were identified, including genes encoding flagella. According to our results, of all the genes mutated, mutations in flagella genes (flgA, flgC, flgK, flhB) of S. Kentucky lead to the most impaired attachment characteristics. This study indicated that attachment of *salmonella* to the surface of broiler skin can be a multifactorial process in which flagella play an important role.

080
CTX-M-type extended spectrum β-lactamase genes in *Salmonella* spp. from livestock clinical diagnostic submissions in the US
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Extended-spectrum cephalosporin drugs are approved for veterinary use in livestock worldwide. In the US, extended activity formulations are frequently applied prophylactically to intensively managed livestock housed in population-dense environments conducive to the exchange of enteric flora. Bacterial resistance to these drugs is conferred by β-lactamase production which is encoded by genes such as $bla_{\text{CTX-M}}$. The widespread dissemination of *Salmonella* spp. carrying $bla_{\text{CTX-M}}$ would pose a threat to animal health and well-being, and their zoonotic food-borne transmission would pose a public health concern. We screened 2034 *Salmonella* clinical diagnostic submissions to the USDA APHIS NVSL between October 2010 and June 2011 for the $bla_{\text{CTX-M}}$ phenotype. We identified 12 (0.6%) *Salmonella* spp. isolates carrying $bla_{\text{CTX-M}}$ on mobile plasmids. We found 6 of 88 (6.8 %) turkey diagnostic submissions carrying $bla_{\text{CTX-M}}$. Of these, one *S.* Bredeney carried $bla_{\text{CTX-M-1}}$ on an IncN plasmid. The remaining 5 turkey isolates were *S.* Ouakam received from March to May 2011 originating from 3 US states carrying $bla_{\text{CTX-M-1}}$ on IncN plasmids. One additional *S.* Ouakam carrying $bla_{\text{CTX-M-1}}$ was identified among submissions received August 2011. One of 940 (0.1 %) swine diagnostic submission was a rough O:d:e,n,z15 isolate carrying $bla_{\text{CTX-M-1}}$ on an IncN plasmid. We found 5 of 143 (3.5 %) equine submissions all from one state between November 2010 and March 2011 were *S.* Anatum, one carried $bla_{\text{CTX-M-1}}$ on an IncN plasmid and the remaining 4 carried $bla_{\text{CTX-M-1}}$ on IncI1 plasmids. All IncN plasmids were ST1 which has been reported to be epidemic in Europe, indicating pandemic dissemination of this plasmid. None of the 581 cattle, 83 chicken, or 199 submissions from other species carried $bla_{\text{CTX-M}}$. The emergence of multiple *Salmonella* spp. carrying $bla_{\text{CTX-M}}$ in diverse US livestock populations is an important concern for both animal and public health.

081
Molecular characterization of the monophasic and non-motile variants of *Salmonella enterica* serotype Typhimurium
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Salmonella enterica serotype Typhimurium variant strains, lacking one or both flagellar phases have been reported worldwide. The monophasic S. 1,4,[5],12:i:-variant has emerged in the past few years and has become one of the most frequently encountered in many countries. In contrast monophasic S. 1,4,[5],12:-:1,2 and non-motile S. 1,4,[5],12:-:-strains are rarely described. By investigating the presence of seven molecular markers by real-time PCR, this study identifies and delineates monophasic S. 1,4,[5],12:i:- (n=90), S. 1,4,[5],12:-:1,2 (n=25), non-motile S. 1,4,[5],12:-:- (n=17) strains and serotype Typhimurium strains (n=124) collected through between 2001 and 2010 in France. Three markers are commonly detected in serotype Typhimurium and in all variant strains: STM2757, mdh and fliA-B. Monophasic S. 1,4,[5],12:i:- are genotypically confirmed by the absence of the fljB , fljA and hin genes. Nevertheless 13 of them (14.5%) are positive for these three genes, revealing monophasic strains named "inconsistent" as previously described. All non-motile 1,4,[5],12:-:- strains displayed the fliC, fljA, fljB and hin genes. The fliC gene is detected in 88% of monophasic S. 1,4,[5],12:-:1,2 strains. The combination of the seven markers enables to recognize eleven different genotypes within the S. 1,4,[5],12:i:- collection. Among them, a specific genotype could be assigned to each of the previously described Spanish and US clone. Based on this molecular approach, 71% of the French S. 1,4,[5],12:i:- collection have been assigned to the Spanish clone and only 2% to the US one. This study highlights the usefulness of these molecular markers and genotypes to identify lineages, especially among the epidemiologically important monophasic S. 1,4,[5],12:i:- variant.

082

Multi-level analysis of *Campylobacter* flock status at post-chill and risk factors within the grow-out environment

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In this study, we investigated risk factors in the grow-out environment that were associated with the likelihood of *Campylobacter* presence on broilers at post-chill. Sampling was conducted in two broiler companies located in three southeastern states, which encompassed 9 complexes, 36 farms, and 72 flocks. Surveys were conducted which evaluated characteristics of the location and layout of the farm, house construction, equipment used in the house, visitation and biosecurity practices, other animal species on the farm, rodent and insect control, litter management, and sanitation. Multi-level-mixed model logistic regression was used to model the relationship between potential risk factors and the probability of Campylobacter presence at post-chill. Of the 350 potential risk factors evaluated, 32 were found to be associated with the presence of Campylobacter at post-chill. These included factors involving litter management, housing conditions and surroundings, farm biosecurity, and sanitation of equipment used.

083

Serotype distribution and antimicrobial resistance profiles of Salmonella, E. coli, and Campylobacter isolates obtained from three broiler production systems in Ontario

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Antimicrobial use in broiler chicken flocks has previously been identified as a risk factor in the development of antimicrobial resistant pathogens that can be transferred to humans. The study objective was to determine the serotype distribution and antimicrobial resistance profiles of Salmonella, E. coli, and Campylobacter isolates according to production type. Seventy-three conventional, 35 antimicrobial-free, and 7 organically-raised flocks distributed throughout Ontario, Canada were enrolled. Within the last 7 days of the growing period samples collected included; dust on feeders, drinkers and walls; boot socks worn to collect material from the floor, and pooled fecal samples. At the isolate level, the 3 most common Salmonella serotypes obtained from conventional flocks (n=195) were Kentucky (53.3%), Heidelberg (10.7%) and Enteritidis (10.3%); vs. Kentucky (54.6%), Heidelberg (26.1%), and Schwarzengrund (11.4%) from antimicrobial-free flocks (n=88); vs. Kentucky (70.0%), Typhimurium (20.0%), and I:8,20:i:- (10%) from organic flocks (n=10). No resistance of Salmonella isolates to azithromycin, chloramphenicol, nalidixic acid, kanamycin, gentamicin, or ciprofloxacin or of E. coli isolates to ciprofloxacin from any production type was found. Controlling for random effect of producer and adjusting for hatchery source, production and sample type, organic flocks had a significantly increased odds of having a Salmonella isolate resistant to ampicillin (OR: 12.3, P = 0.048) compared to conventional flocks. Antimicrobial-free flocks had significantly decreased odds of having an E. coli isolate resistant to several category II and III antimicrobials compared to conventional flocks. Organic flocks had a significantly decreased odds of having an E. coli isolate resistant to streptomycin (OR: 0.3, P = 0.033) compared to conventional flocks. Among Campylobacter isolates, only resistance to tetracycline was identified. In conclusion, these results suggest that differences exist in the serotype distribution and antimicrobial resistance profiles of Salmonella, E. coli, and Campylobacter among flocks raised under the 3 types of broiler production systems in Ontario.

084

Prevalence and fluorquinolone-susceptibilities of Campylobacter and Salmonella in cattle feces from feedlots that use fluoroquinolone therapy for bovine respiratory disease

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The purpose of this study was to determine the prevalence and fluoroquinolone-susceptibilities of Salmonella and Campylobacter spp. in cattle feces from feedlots that used a fluoroquinolone (enrofloxacin) as first-line therapy for bovine respiratory disease. Twenty fresh, pen-floor fecal samples were collected from each of ten pens of near-harvest cattle from five commercial feedlots. Cattle demographic and antimicrobial use data were collected from feedlot records. 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 were evaluated using the Clinical and Laboratory Standards Institute (CLSI) broth micro-dilution methods and human breakpoints. Overall proportions of positive samples were 380/1,000 (38.0%) for Salmonella and 268/1000 (26.8%) for Campylobacter. Salmonella sample-level prevalence varied from 0.5% (1/200) to 76.5% (153/200) among feedlots and from 0% (0/20) to 100.0% (20/20) among pens. Campylobacter sample-level prevalence ranged from 16.0% (32/200) to 41.5% (83/200) among feedlots and from 0% (0/20) to 60.0% (12/20) among pens. Fluoroquinolone

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treatments per pen ranged from 1 to 103. Most Salmonella (99.7%; 379/380) were susceptible to ciprofloxacin and naladixic acid. Susceptibility testing of Campylobacter and multivariable data analyses are currently underway. Preliminary conclusions are that Salmonella and Campylobacter prevalence is highly variable among feedlots and pens. In addition, it appears that most Salmonella in feces of pre-harvest feedlot cattle are susceptible to fluoroquinolones. Prevalence estimates and results from analyses of antimicrobial use and susceptibility data will assist in further assessments of fluoroquinolone use in feedlot production systems.

085
Temporal changes in antimicrobial resistance within Michigan dairy cows
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Purpose: Little is known about the dynamics of antimicrobial resistance (AMR) in a dairy cow as it goes through different production phases and the impact of treatments over time. The goals of this study were to determine the temporal changes in the quantity of susceptible and resistant *Escherichia coli* to four antimicrobials, as well as to determine if health and/or performance events affected the levels and patterns of AMR in dairy cattle.
Methods: 50 cows on a commercial Michigan dairy herd were enrolled. Ten cows in five different stages of production were selected at random: recently freshened, mid-lactation, late-lactation, far-off and close-up dry cows. These cows were sampled throughout a production cycle. Fecal samples were collected from each cow every two weeks and used for bacteriologic culture of *E. coli* via spiral plating on plain MacConkey agar plates (MAC), as well as MAC plates with Ceftiofur, Ampicillin, Tetracycline, and Ciprofloxacin at the resistance break-points. Plates were incubated at 37^{o}C for 18-24 hours and colonies were counted using an automated colony counter.
Data was analyzed using SAS. Results: Initial findings show that 58% of all samples contained *E. coli* that were resistant to at least 1 of 3 antibiotics. Resistance to ciprofloxacin was not observed. Of the samples with resistant *E. coli*, 35% were resistant to at 2 antibiotics and 8% were resistant to all 3s. Further analysis revealed that quantitative *E. coli* counts were lower in cows in the far-off dry group when compared with lactating and close-up dry/ freshened cows. In addition only 7% of samples had resistant *E. coli* compared with 45% and 40% for lactating cows and close-up dry/recently freshened cows, respectively.
Conclusions: By following cows throughout a production cycle, we can study changes in susceptibility of fecal *E. coli* quantitatively as well as assess if the stage of production, treatments, or seasonality affect the levels and patterns of resistance. Understanding the variation in susceptibility patterns of *E coli* in dairy cows will assist in the design of targeted sampling strategies as well as potential interventions to reduce levels and patterns of AMR in cull cows entering the food chain.

086
Prevalence of pathogenic shiga toxin producing Escherichia coli in dairy cattle and wildlife in Texas
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Purpose:Assess the prevalence of O157 and the "big six" (O25, O45, O103, O111, O121 and O145) non-O157 STEC in dairy cattle and wildlife in Texas over the four seasons.
Methods: Fecal samples from dairy cattle and wildilfe reservoirs have been collected in eastern Texas during the summer months. The fecal samples were enriched in tryptic soy broth and tested for the presence of STEC using a multiplex PCR approach. STEC were isolated from positive enriched culture using an immunomagnetic separation method.
Results: Our preliminary work shows that 53.5 % (n=172) of tested cattle were positive for one or more STEC (O26 - 9.9%, O121 - 2.9%, O145 - 9.9%, and O157 - 30.8%) and 48.7% of wildlife (n=39, 14 birds and 25 small rodents) were positive for one or more STEC (O26 - 7.7%, O45 - 5.5%, O103 - 2.6%, O111 - 2.6%, O145 - 17.9%, and O157 - 12.8%).
Conclusions: These results suggest that dairy cattle and wildlife species might play a key role in the transmission of these pathogenic STEC to humans. Our future work will include studying how seasonal differences influence the prevalence of these STEC in dairy cattle and other food animals and determining the role of insects and wildlife species in the transmission of these STEC to cattle.

087
Epidemiology of Shiga toxin-producing Escherichia coli (STEC) shedding in finishing swine- a descriptive longitudinal study
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Purpose: Shiga toxin-producing Escherichia coli (STEC) are an important public health concern, causing more than 200,000 cases of illness annually in the United States. STEC infections are associated with severe diseases in humans: hemorrhagic colitis (HC) and hemolytic uremic syndrome (HUS). STEC infections are predominantly attributed to food or water contaminated by animal feces. There have been outbreaks and cases of STEC infections in humans associated with pork products; however, little is known about swine STEC to date. The objectives of this descriptive longitudinal study are to describe the epidemiology of STEC shedding in finishing swine and to characterize swine STEC strains.
Methods: This study included three cohorts of pigs from one production company. In each cohort, 50 randomly-selected pigs were individually identified, and fecal samples were collected from each pig every two weeks within the 16 weeks of the finishing period (eight samples/pig). Samples were submitted for STEC detection by enrichment (10 min in TSB, pH 3 followed by incubation for 15 h in modified TSB, pH 8.7 at 42 °C) followed by the polymerase chain reaction (PCR) targeting the Shiga toxin genes (stx) and the intimin protein gene (eae). Shiga toxin gene-positive samples were plated onto ChromAgar STEC. Presumptive STEC isolates were recovered and confirmed. Serotypes and stx gene subtypes were characterized. Results: In general, STEC was detected in at least one sample from 31 out of the 50 pigs in cohort 1, 27 out of the 50 pigs in cohort 2, and 40 out of the 50 pigs in cohort 3. STEC was detected one time or more in over 50 % of the pigs in each cohort. The shedding patterns within the finishing period were similar to outbreak curves. To date, a number of O serogroups and stx gene subtypes have been characterized while complete serotype and virulence gene characterization results are pending. The eae gene was not detected in these swine STEC strains. Conclusions: These data will be critical to fill the current knowledge gaps in swine STEC epidemiology and the association of swine STEC and human diseases. Future molecular epidemiologic studies will be conducted to further characterize these swine STEC strains.

FOOD AND ENVIRONMENTAL SAFETY

088

Escherichia coli O104 is prevalent in feces of feedlot cattle, but isolated strains did not carry genes characteristic of enterohemorrhagic or enteroaggregative pathotype

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Shiga toxin-producing *E. coli* (STEC) O104:H4 was the cause of a large foodborne outbreak of hemorrhagic colitis, with an unusually high incidence of hemolytic uremic syndrome, in Germany and France in 2011. The serotype was not a typical STEC, but a hybrid of STEC and enteroaggregative *E. coli* pathotypes that carried genes for Shiga toxin 2 (*stx*2), tellurite resistance (*ter*D), and enteroaggregative fimbrial adhesin (*agg*A), but lacked intimin (*eae*) and enterohemolysin (*ehx*A). Because cattle are known reservoirs of STEC, we investigated fecal carriage of *E. coli* O104 in feedlot cattle. A total of 168 fecal samples (24 per feedlot) were collected from seven feedlots, enriched in *E. coli* broth at 40 C for 6 hours and DNA was extracted from samples before and after enrichment. DNA was subjected to a multiplex PCR (mPCR) that was designed and validated to detect the following 8 genes: *stx*1, *stx*2, *ter*D, *eae*, wzx_{O104} (O104 specific O-antigen flippase), $fliC_{H4}$ (H4 specific flagella), *ehx*A and *agg*A. The prevalence of wzx_{O104} in fecal samples before and after enrichment were 3/168 (1.8%) and 45/168 (26.8%), respectively. None of the fecal samples was positive for *agg*A gene. Enriched samples positive for wzx_{O104} (n=45) were plated on several selective media (MacConkey, CHROMagar and Rainbow) and ten presumptive *E. coli* colonies were picked from each medium, cultured and tested by the 8-gene mPCR. Although 26.8 % (45/168) of fecal samples were PCR-positive for O104, only 7 isolates were confirmed as serogroup O104. All 7 isolates were negative for genes that code for Shiga toxins, H4, intimin, and enteroaggregative adhesin, but five isolates were positive for *ter*D and *ehx*A. The lack of a suitable selective medium for phenotypic selection of O104 may have limited our ability to recover more isolates. Our results suggest that *E. coli* O104 is prevalent in cattle feces, but the strains do not appear to carry genes characteristic of the virulent hybrid strain.

089

Antibiotic use versus antibiotic resistance profiles of commensal *E. coli* in beef cattle: explaining their association via bacterial growth parameters

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Tetracycline genes and their related plasmids can be associated with varying levels of multidrug resistance. In the absence of selection pressures, the relative fitness of strains harboring these traits results in varying prevalence of the associated phenotypic profiles. Introduction of selection pressures will improve the chances for particular resistance profiles that exhibit growth advantages in the presence of the antibiotic, both *in vitro* and *in vivo*. *E. coli* isolates were obtained from a related study examining the effects of feeding chlortetracycline following ceftiofur treatment with differential proportions of treated and untreated cattle. Fecal samples were collected every other day for 26 days and frozen. Thawed samples from Days 0, 4, 12, and 26 were later plated onto MacConkey agar. Three *E. coli* colonies were isolated from each sample. Microbroth dilution was used to measure MIC values for the NARMS panel of antibiotics for each isolate. Qualitative gene detection for each isolate was performed for *tet*A and *tet*B and $bla_{cmy\text{-}2}$ genes. A Bioscreen turbidimeter generated pair-wise growth curves of all 1056 isolates in MacConkey broth, with and without tetracycline (16 µg/ml). Growth parameters for maximum growth rate (µ), time at max growth rate (λ), and peak optic density were calculated for each growth curve ($OD_{[[Unsupported Character - Codename]]max}$). Paired endpoint parameters were analyzed using MANOVA to explore how known isolate properties affect growth fitness. When tetracycline was included in broth, growth presence closely matched suggested resistance data from both MIC and genotypic data. General differences between the pairwise curves were a lower µ, later λ, and lower $OD_{[[Unsupported Character - Codename]]max}$. Multidrug resistant isolates exhibited faster growth rates and reached their maximum growth rate earlier than singly resistant isolates when antibiotics were applied to animals ($P < 0.001$). The data suggest that *E. coli* with multidrug resistance, including tetracycline, may exhibit relatively better growth fitness than *E. coli* resistant to tetracycline alone when under the selective pressure of tetracycline applied to cattle.

090

Commercial evaluation of an SPR-containing *Escherichia coli* bacterial extract vaccine

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Purpose: To evaluate 1) a vaccine containing siderophore receptors and porin proteins (SRP) as an aid to control *E. coli* O157 and 2) its impact on animal health and performance.
Methods: 9,097 animals were allocated to 76 pens (38replicates, 2 pens each) across 8 feedlots. One pen per replicate was allocated to the vaccinate cohort and other to the control cohort. Treatment was a3-dose regimen of an SRP-based vaccine. Cattle were harvested from 27MAY2011 to 27OCT2011. Rectal swabs were collected from 20 animals per cohort at harvest. Real-time PCR (RTi-PCR) was used to detect *stx*, *eae* and genes encoding serogroups O26, O111, O121, O145, O45, and O103. Swab enrichments were stored at -20C prior to culture, thawed at room temperature and 1ml aliquots were transferred into STEC-EB and incubated at 41C. Immunomagnetic separation was performed for O26, O111, O145, and O103 then plated onto Possé agar. Up to 10 colonies were selected per plate and subjected to PCR for O-group determination and presence of *stx* and *eae*.
Results: Audits identified divergence from protocol in 2 feedlots; thus, data from 6 lots, 31 replicates, and 6,803 animals were analyzed. Prevalence of *E. coli* O157 was 12%. *Wzx* genes for O26, O111, O121, O145, O45, and O103 were detected in 49.5, 9.5, 34.9, 80.4, 57.9 and 77.1% of enrichments, respectively. Prevalence of E. coli O157 varied across cohorts but this association varied by a poorly defined covariate which was aggregated within a single yard. Within this yard, no vaccine efficacy (VE) was detected. For the remained 5 yards (without this covariate) VE averaged 52% (P=0.03) over time. Within these 5 yards, *E. coli* O157 was 2 and 4 times more likely to be detected in any swab (70% vs 35%; P=0.04) or a random pool of 5 swabs (60 vs 15%; P=0.01) of control cattle relative to vaccinates, respectively. No effect of vaccine regimen was detected (P>0.20) on health, performance, or carcass characteristics. Serotype recovery data will be presented.

090 (continued)
Conclusions: These data support the efficacy of this SRP-based vaccine as an aid in the control of E. coli O157; further research is needed to better understand yard-level conditions needed for vaccine success.

091
Evaluation of plasmid stability in green fluorescent protein-labeled Escherichia coli O157 in a non-selective, nutrient deficient environment
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Green fluorescent protein (GFP)-labeled *Escherichia coli* O157 is used in many survival and persistence investigations. The labeling of these bacteria may be accomplished by the transformation of GFP plasmids into the bacteria. These plasmids also code for ampicillin resistance which induces the bacterial maintenance of the plasmid when the bacteria are grown in media containing ampicillin. This study investigates the stability of these GFP-labeled bacteria when removed from an ampicillin environment and placed in nutrient-deficient environment similar to that experienced during stationary phase of growth.
Three isolates of a transformed strain of *E. coli* O157, originally isolated from a research feedlot, was used in this study. The isolates were streaked onto TSA plates containing 50µg/ml ampicillin (Ap50) and the most strongly fluorescing colonies were used to inoculate LB Ap50 broth. After incubation, the inoculum was centrifuged and the sediment washed three times in phosphate buffered saline (PBS) to remove any media and antibiotic and resuspended in PBS. Aliquots of the resuspended solutions were placed in microcentrifuge tubes and incubated until the sampling date. The aliquots were diluted and surface plated onto LB and the number of fluorescing and non-fluorescing colonies were counted under UV light. The non-fluorescing colonies were confirmed as *E. coli* O157 by latex agglutination.
At 24 hours post removal (hpr) from the antibiotic environment, the number of non-fluorescing *E. coli* O157 colonies began to increase. By 96 hpr, the number of non-fluorescing *E. coli* O157 colonies was approximately the same as the number of fluorescing *E. coli* O157 colonies. After which, there was a downward trend in the number of both colony types. At 336 hpr, the number of non-fluorescing colonies was approximately 2 log greater than the number of fluorescing colonies. The experiment was repeated and similar results were obtained.
The results demonstrate that once removed from an antibiotic environment, GFP-labeled bacteria do not accurately reflect the actual bacterial population size. To obtain an accurate population count, other methods of labeling bacteria should be employed.

092
Effect of flavophospholipol and environment on antimicrobial resistance in beef cattle.
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Introduction: Incorporation of flavophospholipol (FPL) in production animal systems has been proposed to decrease antimicrobial resistance of plasmid harboring bacteria.
Objective: To field-test practical interventions designed to effectively manage resistance levels in production as well as near-slaughter phases of beef cattle.
Methods: The study implemented a 2 by 2 factorial design with environment (feedlot versus extensive) and FPL administration as the 2 main effects. Feeder steers (n=80) were comingled upon arrival and allowed a 14-day adjustment period. Ceftiofur (ceft) crystalline free acid was administered (6.6 mg/kg) and fecal pen floor samples were collected on day -7. Day 0 cattle were randomly assigned to cohorts; typical feedlot environment with or without FPL or an extensive, non-feedlot environment (1 animal/acre) with or without FPL (8 pens/10 cattle each). FPL was fed at 20 mg/head/day from day 0 to day 14 to appropriate cohorts. Fecal rectal grab samples were collected on days 0, 7, and 14. Day 21 soil, water and feed samples were collected. Fresh feces were diluted (10 g into 90 ml tryptic soy broth) and spiral plated onto MacConkey (MAC) agar, MAC agar containing 8 µg/mL ceft, and MAC agar containing 16 µg/mL tetracycline (tet). Colonies were counted and estimates of cfu/g of feces were calculated.
Results: Unadjusted concentration of non-type specific *E. coli* at day 0 was 6.0 log10 cfu/g averaged across treatment groups. Averaged across FPL administration, the concentration of *E. coli*/g feces was 0.36 log10 cfu/g feces greater in feedlot cattle compared to extensively-housed cattle across days 7 to 14. Ceft-resistant *E. coli* appeared to decrease from day 0 to day 7 followed by a slight increase for all treatment groups from day 7 to day 14. Extensive cohorts, averaged across FPL administration, had 1.3 log10 cfu/g lower concentration of ceft-resistant *E. coli* than feedlot cattle across days 7 to 14. Tet-resistant *E. coli* in extensively housed cohorts appeared to decline across all sampling days.
Conclusion: These data suggest that environmental factors may potentially be more influential than treatment with FPL in modifying the susceptibility of gut flora of beef cattle.

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Discovery of novel alternatives to antibiotic growth promoter to protect food safety
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Antibiotic growth promoters (AGPs) have been used as feed additives to improve average body weight gain and feed efficiency in food animals for more than five decades. However, there is a worldwide trend to limit AGP use to protect food safety and public health, which raises an urgent need to discover effective alternatives to AGPs. The growth promoting effect of AGPs has been shown to be highly correlated with the decreased activity of intestinal bile salt hydrolase (BSH), an enzyme that is produced by various gut microflora and involved in host lipid metabolism. Thus, BSH inhibitors are likely promising feed additives to AGPs to improve animal growth performance. In this study, the genome of Lactobacillus salivarius NRRL B-30514, a BSH-producing strain isolated from chicken, was sequenced by 454 GS FLX sequencer. A BSH gene identified by genome analysis was cloned and expressed in an E. coli expression system for enzymatic analyses. The BSH displayed efficient hydrolysis activity for both glycoconjugated and tauroconjugated bile salts, with slightly higher catalytic efficiencies (kcat/Km) on glycoconjugated bile salts. The optimal pH and temperature for the BSH activity were 5.5 and 41oC, respectively. Examination of a panel of dietary compounds using the purified BSH identified some potent BSH inhibitors, in which copper and zinc have been recently demonstrated to promote feed digestion and body weight gain of different food animals. Together, this study identified and characterized a BSH with broad substrate specificity from a chicken L. salivarius strain and established a solid platform for us to discover novel BSH inhibitors, the promising feed additives to replace AGPs for enhancing the productivity and sustainability of food animals.

FOOD AND ENVIRONMENTAL SAFETY

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Prevalence of transferable copper resistance gene, *tcrB*, in fecal enterococci of feedlot cattle fed diets supplemented with copper

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Purpose: Copper, an essential micronutrient, is supplemented at elevated level in the diet for growth promotion in cattle. Enterococci acquire copper resistance, plasmid-borne transferable copper resistance (*tcrB*) gene which also carries genes form acrolide *erm*(B) and glycopeptide (*vanA*).resistance. Our objectives were to determine the prevalence of *tcrB*-positive fecal enterococci of cattle fed diets supplemented with copper.
Methods: The study consisted of261 crossbred yearling heifers, assigned randomly to a 2×2 factorial arrangement of treatments of dietary copper and linpro. Fecal samples were collected on days 116 and 132. Two enterococcal isolates were obtained from each sample and tested for *tcrB* gene by PCR. All the *tcrB*-positive and an equal number of *tcrB*-negative isolates matched by pen, date, and treatment were also tested for both *erm*(B), *tet*(M), *vanA*, and *vanB* genes, presence of virulence genes, transferability, and MIC determinations for copper, tetracycline, tylosin, and vancomycin. Clonality determinations of *tcrB*-positive were done by multi-locus variable number tandem repeat analysis (MLVA).
Results: The prevalence of *tcrB*-positive enterococci was higher among the cattle fed diets supplemented with copper (6.9%; 20/288) compared to control(0.7%; 2/288). Both *tcrB*-positive and *tcrB*-negative isolates also carried *tet*(M) and *erm*(B) genes and were phenotypically resistant to tetracycline and tylosin, respectively. All were negative for *van* and virulence genes. The median copper MIC's for *tcrB*-positiveand *tcrB*-negative enterococci were 22 mM and 4 mM, respectively. The transferability of the *tcrB* gene was demonstrated by filter mating assay. Southern blot hybridization demonstrated the presence of *tcrB* gene on a ~175 kb size plasmid. The MLVA analysis revealed the presence of genetically diverse and heterogeneous population of enterococci.
Conclusions: Copper supplementation selected for resistant strains. The results suggest potential association between copper resistance and tetracycline and tylosin resistances and also the possibility of enterococci to transfer these resistance genes to other enterococci in the gut.

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An agent-based model to assess the potential effects of vaccines in *Escherichia coli* O157 shedding and transmission in feedlots

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Healthy cattle and their environment are the reservoir for the human pathogen *Escherichia coli* O157. Vaccines based on subunit antigens have been developed to reduce *E. coli* O157 carriage in cattle. Vaccines have multiple biological effects, including reduction on the duration and load of bacterial shedding. Mathematical models are useful tools to assess the overall impact of vaccination in a population. In compartmental models, animals are assumed to have the same level of infectiousness (i.e., bacteria load shed) during their infectious period, which is often assumed to be exponentially distributed. In addition, the different vaccine effects are often considered independent (i.e., a vaccine reduces the duration of the infectious period independently that its effects on the level of infectiousness). These assumptions may lead to incorrect estimates of the overall vaccine effects. Agent-based models (ABM) can overcome some of the limitations of compartmental models by relaxing the above assumptions. Our objective is to evaluate the potential vaccine effects on *E. coli* O157 transmission in feedlots using an ABM that integrates individual animal data on temporal fecal shedding dynamics with pen-level *E. coli* O157 transmission. We evaluated several scenarios that differed in the effect of vaccines on the reduction of bacterial load shedding and the timing between pathogen introduction and vaccination. Reducing the bacterial load to 30% reduced the infection prevalence, and decreased the duration of the outbreak. Prevalence was sensitive to the timing between introduction of the pathogen and vaccination. Vaccination has the potential to reduce *E. coli* transmission and bacterial load substantially during the entire feedlot period if administered early.

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The impact of vaccination and post-harvest intervention failures on beef carcass contamination with E. coli O157

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Vaccination of cattle has been effective in reducing the fecal prevalence and concentration of *Escherichia coli* O157, yet the impact of vaccination on carcass prevalence and contamination has not been evaluated. Understanding the added value of this pre-harvest intervention strategy in different production scenarios is essential. Our objective was to model the effects of vaccination on pre-chill beef cattle carcass contamination with *E. coli* O157 in the presence of industry-standard post-harvest interventions, and at times when post-harvest interventions fail. We constructed a risk assessment using @Risk in Microsoft Excel. Parameter distributions were developed from the existing scientific literature and incorporated into a Monte-Carlo framework. The prevalence and concentration of *E. coli* O157 on beef cattle carcasses were conditional on fecal and high shedder prevalence, transportation and lairage, the relationship between high shedders and hide contamination, hide to carcass bacterial transfer, and in-plant hide and carcass interventions. We assessed the prevalence and concentration of *E. coli* O157 on carcasses based on a standard truckload of 40 head of cattle. Multiple scenarios were evaluated and each scenario was run for 150,000 iterations. Our results indicate the prevalence of *E. coli* O157 on beef carcasses decreased from 4.0 to 3.3% when vaccination reduced fecal prevalence and proportion of high shedders. When we incorporated a vaccine effect on hide prevalence and concentration,carcass prevalence was <1%. The concentration of *E. coli* O157 on carcasses was also reduced by vaccination. These trends were equally evident when hide wash, hide to carcass transfer ratio, and carcass wash efficacy parameters were set to simulate post-harvest intervention failures. Our results indicate reduced carcass contamination of *E. coli* O157 when vaccination in used pre-harvest; the majority of the impact is due to effects on hide prevalence and concentration, however data are not available on the effect of vaccine on hide contamination. Further research is needed to fully evaluate the value of vaccination.

FOOD AND ENVIRONMENTAL SAFETY

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Development of a loop-mediated isothermal amplification assay for point-of-need detection of *Escherichia coli*

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Purpose: *Escherichia coli* in food and water is evidence of fecal contamination from warm-blooded animals and indicates that pathogens may be present. Assays to identify *E. coli* and other fecal indicator bacteria (FIB) at the point-of-need are typically limited to slow culture based techniques. Molecular assays can accelerate the detection of FIB, but currently these assays are not conducive to point-of-need applications. Therefore, new molecular detection technologies are needed for FIB identification. Here, we utilize filtration based concentration of bacteria and describe a field-capable molecular detection platform to rapidly identify *E. coli* in irrigation water and seawater. This platform relies on loop-mediated isothermal amplification (LAMP) and field-ready instrumentation to monitor the LAMP reactions.
Methods: A LAMP assay was developed targeting *ecpA*, the major pilus subunit of the *E. coli* common pilus (ECP), and evaluated with genomic DNA from 30 isolates of pathogenic and non-pathogenic *E. coli* (representing O157:H7, non-O157 Shiga toxin-producing serotypes, and environmental isolates). The efficacy of the LAMP reaction was then tested in contaminated irrigation and seawater. Water samples containing spiked or naturally present *E. coli* were filtered through disposable inline filters (DIF) to concentrate bacteria, field-based nucleic acid isolation was performed, and *ecpA* detected using LAMP.
Results: LAMP-*ecpA* reactions reliably detected 1 pg of genomic DNA from all isolates tested, in less than 50 minutes. Detection times were dependent on the concentration of template DNA (100 ng of DNA was detectable in less than 12 minutes), thus allowing for semi-quantitative estimates of bacterial contamination. Further, LAMP detection of as few as 0.01 CFU/ml *E. coli* was successful in filtrates of seawater and spiked irrigation water, and the entire procedure was completed in 2 hours.
Conclusions: These results demonstrate the feasibility of utilizing the ECP of *E. coli* as a diagnostic target, and the studies presented here provide the foundation for the development of rapid, point-of-need, LAMP assays to evaluate fecal contamination of food and water.

098

Evaluating on-farm interventions to reduce antimicrobial resistance in enteric commensal *Escherichia coli* of cattle with mathematical modeling

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Purpose: Numerous commensal enteric bacteria of food animals serve as a reservoir of plasmidic antimicrobial-resistance (AMR) genes, presenting a food safety concern. There is limited quantitative understanding of the impacts of antimicrobial usage or candidate interventions on plasmid-mediated AMR in these commensals. Methods: We developed deterministic mathematical models of plasmid-mediated resistance of beef cattle *Escherichia coli* (*E. coli*) to the cephalosporin ceftiofur and investigated what factors had an impact on resistance dynamics, both in the absence of antimicrobial usage and during parenteral ceftiofur therapy. Results: The models showed that a fraction of ceftiofur-resistant enteric *E. coli* might persist in the absence of antimicrobial usage. The persistence was influenced by the rate of plasmid transfer and the number of enteric *E. coli*, the rate of replacement of enteric *E. coli* by ingestion, and the frequency of resistance in ingesta. The latter is largely influenced by *E. coli* populations in the animal's water supply and the extent of *E. coli* turn-over between the on-farm animate and non-animate habitats. In a treated animal, resistant enteric *E. coli* expanded in absolute number and relative frequency during parenteral ceftiofur therapy, irrespective of drug formulation. However, the long-term post-therapy outcome again depended on the ecological parameters of *E. coli* quantity, circulation, and plasmid transfer. Most instrumental in reducing ceftiofur resistance in enteric *E. coli* by cattle slaughter age were interventions that either induce "plasmid cure" or reduce the rate of plasmid transfer or the number of *E. coli* in its enteric habitat. Also important were the dynamics of *E. coli* turn-over between its animate and non-animate habitats, for which little empirical information exists. Conclusions: Differences in the turn-over dynamics and in *E. coli* contamination of animal water and feed supplies may be underlying the variations in AMR frequency between cattle rearing systems.

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Impact of feeding distillers grain-based diets on the colonic microbial community structure of cattle

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With the increased use of corn for the production of ethanol, there is an increased supply of the distillation by-products to be used as feed for agricultural animals. Distillers grains (DG) provide more protein, fat, and fiber than more traditional corn diets. Several researchers have indicated that diets rich in DG increase the shedding of Escherichia coli O157 by cattle. The different dietary composition of DG may influence the shedding dynamics of E. coli O157 from cattle by modifying the colonic microflora.
To better understand the effects DG have on the ecology of the colonic microflora, analyses of the microbial community structure of cattle fed different diets was performed. Swab samples were taken from the recto-anal mucosal junction of 42 cattle fed DG the entire length of the study and 42 cattle switched to a corn-based feed after 150 days on DG. Samples were taken 6 days prior to the diet change, the day of the switch, and 8, 15, and 22 days after the diet change. Comparisons of the microbial community structure of corn-fed and DG-fed cattle were performed using discriminant analysis of terminal restriction fragment length polymorphisms (TRFs) of amplified 16S ribosomal gene sequences.
These data showed that microbial population profiles between corn and DG fed steers were influenced by diet as well as time on diet. Eleven TRFs, ranging in size from 125 bp to 547 bp, contributed to the discrimination of the DG-fed steers from the corn-fed steers. Sequencing of these fragments is currently underway and may led to the identity of potentially functional bacterial species that alter the shedding dynamics of E. coli O157.

FOOD AND ENVIRONMENTAL SAFETY

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An analysis of foodborne illness risk factor violations and bacterial load in restaurant food preparation areas.

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Previous studies have concluded that restaurant inspection alone may not prevent a foodborne illness outbreak and that local health department inspection procedures may need to be changed. The objective of the study was to determine whether Champaign-Urbana Public Health District (CUPHD) Foodborne Illness Risk Factor Violation Scores are associated with bacterial load within the food preparation areas of restaurant kitchens. A case-control study was conducted to test the hypotheses that case restaurants have significantly higher coliform, Staphylococcus, and total aerobic bacteria counts on food preparation surfaces when compared to control restaurants. A database of CUPHD restaurant inspection score reports from 2009-2010 was used to identify case and control restaurants. Case restaurants were those whose mean violation scores from the previous 3 inspections were greater than 1 standard deviation above the overall mean. Control restaurants were those whose mean scores from the previous 3 inspections were greater than 1 standard deviation below the overall mean. A total of 97 restaurants were invited to participate in the study and 44 restaurants were enrolled. Samples were obtained by direct contact inoculation using Petri-film™ media plates on 4 surfaces in the restaurant kitchen. Samples were collected in duplicate. A total of 1,008 total plates were inoculated and read. Case restaurants were 8 times more likely to have above average Staphylococcus counts compared to control restaurants (OR 8.31, 95% C.I. = 0.92-74.89). When compared to control restaurants, case restaurants were almost 7 times more likely to have a cutting board that was coliform positive (OR 6.86, 95% CI = 1.50-33.92), and 5.4 times more likely to have a refrigerator surface that was coliform positive (95% C.I. =1.19-26.08). Levels of bacterial contamination by these organisms may serve as indicators of conditions that may be suitable for the persistence of pathogens capable of causing foodborne illness. These findings can be used to provide data to support the development of future health department policies, educational programs, or inspection changes for food safety.

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In vitro effect of deoxynivalenol (DON) mycotoxin on porcine circovirus type 2 (PCV2) replication and cytopathic effect.

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Deoxynivalenol (DON) is a trichothecene mycotoxin produced by *fusarium spp*. DON is an important food safety issue because it is the most commonly detected mycotoxins of cereal grains. Among monogastric farm animals, pigs are the most susceptible to DON because it markedly reduces feed intake and decreases weight gain, even at low feed contamination. Investigations of host resistance, cell-mediated immune response and humoral immunity indicate that DON is both immunostimulatory and immunosuppressive depending on dose frequency and duration of exposure as well as type of functional immune assay. The objective of this study was to investigate *in vitro* effect of the DON mycotoxin on replication and cythopathic effect of porcine circovirus (PCV) in newborn pig tracheal permissive cell line (NPTr). Non-infected cells and cells infected with different subtypes of PCV (PCV2a and PCV2b) were treated with increasing concentration of DON mycotoxin (0, 70, 140, 280, 560, 1200 ng/ml). Cell survival was evaluated by determining the number of viable cells with tetrazolium compound (CellTiter, promega) after 72hrs of infection. Cell mortality was also evaluated by measuring LDH release. Finally, virus titration was performed after 72 hrs of infection by qPCR. DON significantly affects the survival of non infected cells at the concentration of 280 ng/ml or higher. At same concentration, DON significantly increased the mortality of PCV2b infected cells. Unlike PCV2b, addition of DON at equivalent concentration decreases the mortality of PCV2a infected cells. Immunofluorescence and qPCR analyses indicate that DON mycotoxin increases PCV2b but decreases PCV2a replications. DON mycotoxin had a significant effect on the viability of PCV infected cells and on PCV replication, in a dose dependant manner and a genotype dependant manner. However, more experiments will be needed to determine the *in vivo* significance of these results.

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A one health approach to public health issues in Ghana

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The University of Illinois Global Health Initiative aims to: coalesce a research community around global health issues on the Urbana-Champaign campus; create capacity for future interdisciplinary work on global health research; and to provide first-hand exposure to global health issues for graduate students and faculty. In January, 2012, a multi-disciplinary group of students and faculty from the University of Illinois traveled to Ghana to observe healthcare in a resource-limited setting and begin planning for on-going research and teaching activities in the region. Like much of the developing world, Ghana faces significant public health challenges. Infectious diseases including malaria, tuberculosis, childhood diarrheal disease and HIV/AIDS, dominate the case load in many hospitals and health centers. As a result, the infant (50 per 1,000) and child (74 per 1,000) mortality rates are elevated and life expectancy is 64 years of age. Low access to health services, and to safe water and sanitation, high incidence of malaria and malnutrition have all been identified as underlying factors among the main causes of childhood mortality in the region. Major threats to public health in Ghana include: Infectious disease among the human population, access to clean drinking water, access to sanitary waste disposal and inadequate nutrition. One limitation of most projects that seek to improve public health by treating tuberculosis and other zoonotic diseases is that they often fail to address the role of wildlife and domestic animal reservoirs in the maintenance of these diseases in the human population. This presentation will focus on the intersections between human, animal, and ecosystem health in the Central Region of Ghana. Sustainable improvements in public health will require the establishment of health, education, and infrastructure projects that address health in the human, domestic animal, and wildlife populations that share the ecosystem.

GASTROENTERIC DISEASES

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Unveiling the mysteries of iron regulation in *Mycobacterium avium* subspecies *paratuberculosis*

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Mycobacterium avium subsp. *paratuberculosis* (*MAP*), the causative agent of Johne's disease (JD), requires mycobactin supplementation for growth in laboratory media. This unique iron requirement makes *MAP* fastidious often requiring eight to sixteen weeks to produce colonies - a major hurdle in the timely implementation of JD control measures. My laboratory has characterized IdeR as a transcriptional regulator of mycobactin synthesis and iron storage genes. Furthermore, we have made multiple discoveries - (1) corrected the orientation of *ideR* on the MAP genome; (2) identification of a novel operon carrying genes encoding ESX-3/type VII secretory system; (3) variation in *ideR* regulation of iron acquisition and storage between the type I and type II *MAP* strains; and (4) the identification of *in-vivo* upregulation of *fur* located in the *MAP*-specific pathogenicity island. Identification of upregulation of a novel *fur* element *in-vivo* suggests that MAP likely employs alternate regulatory pathways, unlike other pathogenic mycobacteria. I will present key findings in iron regulatory pathways employed by MAP and their potential role in transepithelial migration and macrophage persistence.

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A novel vaccine candidate protecting cattle against diarrhea caused by enterotoxigenic Escherichia coli (ETEC) and bovine viral diarrhea virus (BVDV)

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Diarrhea is one of the most important diseases in cattle. Enterotoxigenic Escherichia coli (ETEC) strains expressing K99 (F5) fimbriae and heat-stable type I (STa) enterotoxin are the major cause of diarrhea in calves, while the bovine viral diarrhea virus (BVDV) is a major cause of diarrhea in cattle of all ages. The goal of the work presented here was to create a multivalent vaccine that protects calves against both pathogens. The K99 adhesin and STa enterotoxin are major virulence determinants for ETEC and the E2 glycoprotein is the most immunogenic protein of BVD. We hypothesize that a novel multivalent vaccine consisting of FanC, the major fimbrial subunit of K99, STa and BVDV E2 antigens will induce broad protective immunity. We constructed a genetic fusion protein using the K99 major subunit FanC as a carrier of STa, a B cell epitope and a T cell epitope of the BVDV E2 glycoprotein (with STa & BVDV epitopes were embedded in the FanC major subunit). This fusion proteins were used to test for safety and immunogenicity in a murine model, and to examine vaccine potency. Our data showed that the immunized mice developed anti-K99, anti-STa and anti BVDV antibodies. Moreover, induced antibodies showed neutralization activities against both ETEC and BVDV. This suggests that this novel fusion can be an ideal antigen for developing a broadly protective vaccine against cattle diarrhea in cattle.

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A genetic fusion of enterotoxins of enterotoxigenic Escherichia coli (ETEC) induced broadly antitoxin immunity against ETEC associated diarrhea

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Enterotoxigenic Escherichia coli (ETEC) are the main cause of diarrhea in young pigs (and other livestock animals, & humans as well). Neonatal and post-weaning diarrhea causes significant economic losses to swine producers worldwide. Currently, there is no broadly effective vaccine to protect young pigs against ETEC diarrhea. Enterotoxins, including heat-labile (LT), heat-stable (STa, STb) and shiga-like toxin Stx2e, produced by ETEC strains are the virulence determinants in porcine ETEC-associated diarrhea. These enterotoxins disrupt fluid homeostasis in pig small intestinal epithelial cells that leads to fluid and electrolyte hyper-secretion and diarrhea. A broadly protective antitoxin vaccine that induces host immunity to neutralize enterotoxicity of LT, STa, STb and Stx2e to protect against diarrhea is urgently needed. In this study, we genetically constructed a fusion antigen which consists of antigenic epitopes from LT, STa, STb and Stx2e, and examined safety and immunogenicity of this fusion antigen in a murine model. Mice i.p. immunized with this fusion antigen developed antitoxin antibodies specific to each toxin. Furthermore, induced antitoxin antibodies exhibited neutralizing activities against these enterotoxins. These data indicated this fusion antigen induced broad antitoxin immunity, and suggested this fusion antigen could be used in future development of broadly protective antitoxin vaccines against ETEC diarrhea in pigs.

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Safety and immunogenicity studies of a modified heat-labile toxin (LT) and heat-stable toxin (ST) fusion protein (LTS63K/R192G/L211A-3xSTaA14Q) in a murine model

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Enterotoxigenic Escherichia coli (ETEC) is the leading bacterial cause of diarrhea that results in significant morbidity and mortality in both humans and animals. The virulence factors of ETEC in diarrhea have been well characterized, and these virulence factors become the primary antigen targets in vaccine development. Following the colonization at small intestine, an ETEC strain elaborates heat-stable toxin (ST), heat-labile toxin (LT), or both, to disrupt fluid homeostasis in host small intestinal epithelial cells that causes electrolyte-rich fluid hyper-secretion and diarrhea. Thus, antigens from both toxins must be included in a vaccine. In order to utilize ST and LT as vaccine components, we need to detoxify both enterotoxins for antigen safety. In addition, due to the poor immunogenicity, ST should be linked to a strongly immunogenic carrier protein to induce immune responses. In this study, we constructed a genetic fusion protein consisting of a triple mutated monomeric LTAB peptide (S63K/R192G/ L211A) and three copies of a STa toxoid (A14Q) to explore potential application in ETEC antitoxin vaccine development. Our data showed that this fusion protein had significant reduction in toxicity as it did not stimulate an increase of intracellular cyclic AMP and cyclic GMP levels in T84 cell line, and it induced immune responses in mice. Mice immunized intraperitoneally (IP), intranasally (IN) or sublingually (SL) developed anti-LT and anti-STa antibodies. Moreover, induced anti-LT and anti-STa antibodies were shown to neutralize

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cholera toxin (CT; a homology of LT), and STa toxin in vitro. Results from this study demonstrated that the detoxified LT-STa fusion protein is safe and immunogenic, and can serve as an antigen for a subunit vaccine against ETEC diarrhea.

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Development of a modified live vaccine against enterotoxigenic *Escherichia coli*-associated porcine post-weaning diarrhea
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Post-weaning diarrhea caused by enterotoxigenic *Escherichia coli* strains expressing K88 or F18 and enterotoxins (heat-labile and/or heat-stable toxins) result in significant economic losses to swine producers worldwide. Vaccines inducing anti-adhesin immunity to block adherence of K88 and F18 fimbriae and antitoxin immunity to neutralize LT and ST toxins could broadly protect pigs against ETEC diarrhea. In this study, we genetically fused a K88ac major subunit FaeG peptide, a F18 minor subunit FedF peptide, and the LT_{A2} peptide and the LT_B subunit peptide of LT for fusion protein, and expressed this fusion in a holotoxin-like structure for developing a live vaccine strain. A non-pathogenic *E. coli* strain G58-1 expressing 987P fimbriae was used as the host strain to express and deliver this LT-like fusion antigen. Gnotobiotic piglets orally immunized with this live strain, after inoculation of porcine normal GI flora, developed anti-K88ac, anti-F18 and anti-LT antibodies (IgG, sIgA). In addition, induced antibodies inhibited adherence of ETEC strains expressing K88ac and F18 fimbrial and neutralized the cholera toxin (CT; a homology of LT) *in vitro* assays. Furthermore, all immunized piglets remained healthy after being challenged with diarrheagenic ETEC strain 3030-2(K88ac/LT/STb). In contrast, all control pigs developed severe diarrhea and died within 36 h post-inoculation. Data from this study showed that this modified live strain expressing the holotoxin-like antigen (FaeG-FedF-$LT_{A2:B}$) induced protective anti-K88ac, anti-F18 and anti-LT antibodies, and suggested that this live strain can be developed as a broadly effective vaccine against porcine ETEC diarrhea.

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Glucose significantly affects enterotoxigenic Escherichia coli adherence to intestinal epithelial cells through its effects on heat-labile enterotoxin production
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Previously, heat-labile enterotoxin (LT) was shown to promote the adherence of enterotoxigenic E. coli (ETEC) to intestinal epithelial cells, and in other studies found to have its production stimulated by glucose. The present study was conducted to determine whether ETEC exposure to glucose at different concentrations will affect its ability to adhere to intestinal epithelial cells via its effects on LT production.
A total of 8 wild-type and recombinant isogenic LT+, LT- porcine-origin ETEC strains were grown in Casamino acid yeast extract medium (pH 8.5, 0.25% glucose) from which washed bacteria were used to infect IPEC-J2 cells in cell culture media containing 0, 0.25, 0.5, 1 and 2% glucose, at a multiplicity of infection of 100:1. Infected IPEC-J2 cells were incubated at 37℃, 5% CO_2 for either 2 h (for adherence assay) or 4 h (for cAMP assay), and subsequently plated on LB agar for enumeration. IPEC-J2 cells were pre- or co-incubated with different concentrations of GM1 ganglioside and LT to determine the effect of LT on adherence.
All LT+ strains had higher ETEC adherence to IPEC-J2 cells than did LT- strains. Adherence of the LT- but not the LT+ strains was increased by pre-incubating the IPEC-J2 cells with LT in a dose-dependent manner(P<0.05). Adherence was blocked by GM1 ganglioside also in a dose-dependent manner (P<0.05). LT production, adherence and cAMP levels of infected IPEC-J2 were all at a maximum when LT+ strains were grown with 0.25% glucose. Adherence and cAMP levels of IPEC-J2 cells infected with LT- strains were not affected by the glucose concentration of the medium.
This study demonstrated that maximal LT production by ETEC bacteria occurs in the presence of 0.25% glucose in the culture media, a concentration which promotes ETEC adherence to intestinal epithelial cells. This is the first study demonstrating that glucose (at a specific concentration) in the culture medium, through its effects on LT production, significantly affects bacterial adherence, lending support to the hypothesis that the composition of dietary nutrients in the intestine of the host may directly influence the severity of ETEC infection.

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Laser capture microdissection coupled with RNA-seq analysis to evaluate the transcriptional response of pigs experimentally infected with Lawsonia intracellularis
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Lawsonia intracellularis is the causative agent of proliferative enteropathy (PE), an endemic disease in pigs and an emerging concern in horses. Cell proliferation is directly associated with bacterial replication in the intestinal epithelium, but the molecular basis involved in the host-pathogen interaction during infection is unknown. The present study used laser capture microdissection coupled with RNA-seq technology to characterize the transcriptional response of infected enterocytes in vivo. Five 3-week-old pigs were orally infected with 109 L. intracellularis organisms and five with placebo. At necropsy, ileal tissue samples were collected and frozen sections were stained by immunohistochemistry using Lawsonia-specific antibody. Approximately, 3,000 infected and non-infected enterocytes were microdissected from each pig in the infected and the control groups, respectively. Amplified cDNA was prepared using Ovation® RNA-Seq System and sequenced using Illumina® platform. Comparing the differential expression of 11,778 expressed genes, 119 were significantly induced and 46 were repressed in L. intracellularis¬-infected enterocytes (2 fold-change; p<0.05). Expectedly, cell cycle-associated genes (e.g. cyclin-dependent kinases) were up-regulated in infected cells. However, these cells showed down-regulated expression of various apical membrane transporters (e.g. sodium-dependent glucose transporter) suggesting that this reduced expression in infected enterocytes is compensated by the expression of the facilitated glucose transporter (fold-change=4.02) in the basal membrane. Interferon gamma-induced proteins were up-regulated in infected cells and may be involved in the immune response to intracellular bacteria. Our study is the first report showing cell-specific transcriptional response to Lawsonia infection in vivo. The identification of enterocyte-specific genes differentially expressed between infected and non-infected cells demonstrated the feasibility and relevance of coupling laser microdissection and RNA-seq technology to evaluate gene expression profiles from a specific cell population present in a heterogeneous tissue.

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Development of novel vaccines for mitigation of Campylobacter in poultry
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Purpose: *Campylobacter*, the leading bacterial cause of human enteritis in many industrialized countries, is highly prevalent in poultry. Thus, on-farm control of *Campylobacter* in poultry would reduce the risk of human exposure to this pathogen and have a significant impact on food safety and public health. Vaccination is a promising strategy for control of *Campylobacter* in poultry. To date, vaccines against *Campylobacter* infection are still not available, primarily due to the antigenic complexity of this organism and a lack of understanding of the mechanisms of pathogenesis. Recently, we have provided compelling evidence that outer membrane proteins CmeC and CfrA are feasible *Campylobacter* vaccine candidates. Methods: To further develop effective vaccination strategies, oral live *Salmonella*-vectored vaccine and DNA vaccine were constructed in this study. The USDA licensed live attenuated *Salmonella enterica* serovar Typhimurium vaccine strain χ8914 and vector pYA3493 were used for construction of recombinant *Salmonella* vaccine expressing CmeC or CfrA. Production of desired CmeC or CfrA in the *Salmonella* vaccine strains were confirmed by immunoblotting. To construct DNA vaccine for *in ovo* and intranasal immunization, the *cmeC* and *cfrA* genes were cloned into the eukaryotic expression vector, pCAGGS.
Results: Sequencing of the recombinant vectors confirmed the desired insertion.
Conclusions: In conclusion, two types of *Campylobacter* vaccines were developed in this study, which provides us a solid foundation to evaluate different vaccination regimens for effective mitigation of *Campylobacter* in poultry in the future.

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Comparative pathogenicity of porcine rotavirus group A, B and C in neonatal pigs
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Rotaviruses, containing seven serogroups (A to G), are an important cause of diarrhea in humans and animals. Serogroups A, B and C are frequently identified as a cause of enteric disease and diarrhea in younger pigs. Historically, rotavirus group A has been the main cause of postweaning diarrhea; however, neonatal diarrhea associated with rotavirus group C infection has been increasingly diagnosed. It is believed that rotaviruses in different groups have different biological properties including pathogenicity. The following study was conducted to compare the pathogenicity of rotavirus group A, B and C individually or in combinations in naïve newborn piglets. Forty eight one-day old Cesarean-Derived and Colostrum-Deprived (CDCD) pigs were divided into eight groups. Pigs in each group were challenged with rotavirus that belong to individual group A, B, C or all combinations of group A, B and C. One half the pigs of each group were euthanized at 24 hours post-inoculation (PI) and the remaining at 72 hours PI. Clinical signs were recorded every 12 hours PI. Rectal swabs were obtained before inoculation and then every 12 hours thereafter. Intestinal contents were also collected at necropsy. Swabs and content were tested by PCR for virus shedding and multiple parts of intestine (duodenum, jejunum and ileum, descending colon, cecum) were collected for histopathology. All rotavirus groups appeared to be equally pathogenic to immunologically naïve neonates. Based on the clinical signs, there is no remarkable difference in the magnitude of diarrhea caused by rotavirus group A, B, and C or combinations. The onset of group B and C shedding was earlier than group A; however, rotavirus group A and C were shed longer than group B. In conclusion, all serogroups A, B and C of rotavirus can cause the disease in CDCD neonatal piglets.

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Characterization of porcine group B rotavirus G genotype in the United States reveals substantial genetic diversity
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Abstract Not Available

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Characterization of swine group C rotavirus G genotypes from the United States and Canada reveals a new proposed G genotype
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Initially named pararotavirus, group C rotavirus (RVC) was discovered in a 27-day old nursery pig from an Ohio, United States, displaying clinical signs of diarrhea. While swine RVC infections can appear as both clinical and subclinical, recent discoveries have identified RVC from a substantial number of swineherds in North America with clinical signs of diarrhea. Moreover, discovery of RVC in porcine lung tissue with rotavirus-like lesion by histopathology may suggest a viremic state of RVC similar to group A rotavirus. While recent data on the genetic diversity of RVC G genotypes in North American is unavailable, we investigated the genetic relatedness of porcine RVCs with clinical signs (diarrhea and weight loss) by sequencing the VP7 open reading frame (ORF) of 70 porcine samples from 11 states (USA) and one Canadian providence between 2009-2011. Using the novel sequence data generated in this study, the 89% amino acid genotypes classification cut-off value proposed was modified which identified a new G genotype. While a commercially RVC vaccine is unavailable for pigs in North America, these preliminary characterizations of RVC G genotypes may help guide future vaccine development.

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The effect of climate change on the evolution of food- and waterborne diseases: a systematic review.
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Warming environmental temperatures and increased precipitation may influence the ecology, survival, and transmission of microbial populations, including pathogens that cause human gastroenteric diseases. Although primary research on this subject has been published since the 1980's, the extent to which climate change has affected the epidemiology of zoonotic infections is still poorly understood. In this study, the systematic

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review process was used to identify the available electronically classified research on the prevalence, concentration, transmission, and survival of 30 infectious food- and waterborne pathogens. Search terms included a variety of relevant climate change terms. A total of 5528 papers were identified from seven databases. After de-duplication and screening for relevance, 252 primary research articles, literature reviews, and reports published between 1960-2012 were retained. Approximately half of the articles (120) were classified as primary research. The remaining articles were literature reviews and commentaries. The most common pathogens addressed were Vibrio (90 articles), Cryptosporidium (39) Salmonella (35), E. coli (34). This systematic process of evaluating the literature provides a foundation for understanding the impact of climate change on human disease and a framework to identify current gaps in research knowledge that will facilitate prioritization of future research.

IMMUNOLOGY

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Bovine tuberculosis research: Immune mechanisms relevant to biomedical applications

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Pioneer studies on infectious disease and immunology by Jenner, Pasteur, Koch, Von Behring, Nocard, Roux, and Ehrlich forged a path for the dual-purpose with dual benefit approach, clearly demonstrating the relevance of veterinary studies for biomedical applications. Tuberculosis (TB), primarily due to Mycobacterium tuberculosis in humans and M. bovis in cattle, is an exemplary model for the demonstration of this concept. Early studies with cattle were instrumental in the development of the use of Koch's tuberculin as an in vivo measure of cell-mediated immunity for diagnostic purposes. Calmette and Guerin demonstrated the efficacy of bacille Calmette Guerin (BCG, an M. bovis Nocard strain attenuated by serial passage) in cattle prior to use in humans as a vaccine. The interferon-γ release assay, now widely used for TB diagnosis in humans, was developed for use in the Australian bovine TB eradication program, circa 1990. More recently, experimental infection and vaccine efficacy studies with cattle have demonstrated a correlation of vaccine-elicited central memory (TCM) and IL-17 responses to protective efficacy, robust γδ T cell responses to mycobacterial antigens upon infection, correlation of specific antibody to mycobacterial (antigen) burden and lesion severity, anti-mycobacterial activity of CD4+ T cells via granulysin production, and an association of SIRPα+ cells with ESAT-6:CFP10-elicited multinucleate giant cell formation. Additionally, positive prognostic indicators of bovine TB vaccine efficacy (i.e., responses measured after infection) include: reduced antigen-specific IFN-γ, iNOS, IL-4, and MIP1-α responses; reduced antigen-specific expansion of CD4+ T cells; and a diminished activation profile on T cells within antigen stimulated cultures. Delayed type hypersensitivity and IFN-γ responses generally correlate with infection (i.e., diagnostic) but do not correlate with lesion severity. Comparative immunology studies including partnerships of researchers with veterinary and medical perspectives will continue to provide mutual benefit to TB research in man and animals.

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The role of bovine γδ T cells and their WC1 co-receptor in interacting with bacterial pathogens and promoting vaccine efficacy.

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γδ T cells are critical to immune surveillance and protection since they are found as resident cells in many organs and tissues, including in humans and ruminants, and circulate at substantial numbers in the blood. It is known that γδ T cells contribute to cellular immunity and protection against important pathogens including organizing granulomas in response to Mycobacteria. We have shown that IFNγ-producing bovine γδ T cells bearing the WC1 co-receptor are the major cell population responding in recall responses to Leptospira during the first month following priming by vaccination against serovar Hardjo. To date, successful vaccines largely include those to diseases that only require antibody responses for protection and attempts at creating subunit peptide vaccines to stimulate conventional γδ T cell for cellular immune responses have been mostly unsuccessful. However activation of nonconventional T cells such as γδ T cells that direct adaptive T cell responses has received little attention for improving vaccines because it is not clear how best to prime γδ T cells for recall responses. Annotation of the bovine genome showed there were 13 WC1 molecules coded for by individual genes; the WC1 molecules are distributed among cells to form a number of γδ T cell subsets and that this number is conserved among breeds and individuals as our the gene sequences. Using RNA silencing we have shown that the WC1 co-receptor contributes to the ability of γδ T cells to respond to leptospira. The leptospira-responsive γδ T cells are found within a subset of the serologically-defined WC1.1+ γδ T cell subpopulation and our data indicate that the WC1 molecules expressed act as pattern recognition receptors interacting directly with bacterial components. We are now extending this work to Mycobacteria bovis.

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Characterization of the antigen-specific γδ T cell response following virulent Mycobacterium bovis infection in cattle

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γδ T cells respond to Mycobacterium bovis as evidenced by migration to draining lymph nodes (dLN) in vaccinated or infected cattle and by responding with proliferation and cytokine production in vitro. Bovine γδ T cells express the hybrid pattern recognition/co-receptor WC1, a member of the Scavenger Receptor Cysteine-Rich superfamily. There are 13 WC1 genes, and differential expression of these gene products can be used to divide bovine γδ T cells into several serologically-defined subpopulations. Previous studies suggest that M. bovis responsive γδ T cells are contained within the WC1+ population and, more specifically, within the WC1.1+ subset; however, this evidence is primarily correlative and it remains unknown which of these subsets are directly responding to M. bovis. Further, the conditions and specific antigens required to activate bovine γδ T cells following M. bovis infection remain poorly defined. Therefore, we undertook studies to determine the responsiveness of γδ T cells to mycobacterial antigens and to determine the WC1 subset(s) responding following virulent M. bovis infection in cattle. Here, we demonstrate that circulating γδ T cells respond robustly to both protein and non-protein mycobacterial antigens following M. bovis infection. We observed responses to the protein antigens PPD-B and recombinant ESAT6/CFP-10, a target known to be highly antigenic for CD4 T cells, and

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significant IFNγ production to the non-protein antigens mycolylarabinogalactan peptidoglycan (mAGP) and the glycolipid mannosylated lipoarabinomannan (LAM), both components of the mycobacterial cell wall. This response was both specific and direct, as γδ T cell IFNγ production was observed in the presence of mixed PBMC and when purified γδ T cells were cultured with APCs alone. Interestingly, we observed IFNγ responses by all γδ subsets including the WC1neg, WC1.1+ and WC1.2+ populations in short-term 24-hour cultures. Future studies will be aimed at identifying the WC1 γδ subsets that localize to the lungs and dLN following M. bovis infection, and detailing the cytokine profile, WC1 gene usage and γδ TCR gene usage of responding γδ T cells following stimulation with mycobacterial antigens.

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WC1 functions as a pattern recognition receptor and co-receptor for γδ T cells
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WC1 proteins, exclusively expressed on the surface of bovine T cells, are members of the scavenger receptor cysteine-rich (SRCR) superfamily and are coded by 13 genes. Phosphorylation of a tyrosine in the WC1 cytoplasmic domain upon co-ligation of WC1 and TCR is required for WC1 co-receptor activity. Serologically-defined WC1.1+ γδ T cells specifically respond to the spirochete *Leptospira*; in contrast, WC1.2+ γδ T cells respond to the rickettsiales *Anaplasma*. Silencing via shRNA suggests that only cells expressing the WC1 gene products known as WC1-1, WC1-2 or WC1-3 contribute to the γδ T cell response to the spirochete *Leptospira*, suggesting that WC1 receptors encode antigen specificity. In support of this concept, other closely related SRCR family members, such as DMBT1, CD6, and CD163A, are known to bind to bacteria. Comparing a representative of WC1 proteins expressed by WC1.1+ cells (i.e., WC1-3) to a representative of WC1.2+ cells (i.e., WC1-4), we found that five out of eleven WC1-3 SRCR domains and none of the eleven WC1-4 SRCR domains bound to two *Leptospira* species, *L. borgpetersenii* and *L. interrogans*. Binding by two of the five leptospire-binding WC1-3 SRCR domains is dependent on the temperature at which the leptospires are cultured, suggesting that the WC1-3 SRCR domains do not all bind to the same ligands. Proteinase K digestion or polymyxin B treatment of leptospires does not negatively impact binding, indicating that the ligands are not proteins or lipopolysaccharide (LPS). In contrast, treatment of leptospires with alkaline phosphatase decreased WC1-3 SRCR binding, indicating that the ligand is phosphorylated and may be similar to isopentenyl pyrophosphate (IPP), a non-peptidic γδ T cell ligand and bacterial product whose binding is decreased with dephosphorylation. Mutational analysis of WC1-3 SRCR domains suggests that the active site for bacterial binding is different from that previously reported for other SRCR proteins that bind to bacteria and also that it is affected by single amino acid changes. Taken together, our data suggest that co-ligation of specific WC1 proteins and the γδ TCR by pathogen-associated molecular patterns (PAMPs) induces γδ T cell activation.

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Effector and memory T cell subsets in the response to bovine tuberculosis.
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Long-term (i.e., 14 days) cultured IFN-γ ELISPOT assays of peripheral blood mononuclear cells (PBMC) are used as a correlate of T cell central memory (Tcm) responses in both cattle and humans. With bovine tuberculosis, vaccine-elicited long-term IFN-γ ELISPOT assays have been used as a correlate of protection; however, the phenotype of the IFN-γ producing cells has not been defined. The objective of the present study was to characterize the relative contribution of Tcm, T effector memory (Tem), and T effector cells to IFN-γ production in standard *ex vivo* and long-term (cultured) antigen-specific assays (n = 8) in response to aerosol *M. bovis* infection. PBMCs collected from infected cattle were stimulated with a cocktail of *M. bovis* purified protein derivative (PPDb), rESAT-6:CFP-10 (E:C) and over-lapping peptide cocktails of Tb10.4 and Ag85A for 13 days with periodic addition of fresh media and rIL-2. On day 13, cultured PBMC were re-stimulated with medium alone, E:C, or PPDb with fresh autologous adherant cells for antigen presentation. After overnight stimulation, cells were analyzed for CD4, CD45RO, CD62L, CD44, and CCR7 (rat anti-human CD197) expression via flow cytometry. In response to E:C or PPDb, ~75% of $CD4^+$, IFN-γ^+ cells expressed a Tcm phenotype ($CCR7^+$, $CD62L^{hi}$, $CD45RO^+$) and ~24% expressed a Tem phenotype ($CCR7^-$, $CD62L^{mod}$, $CD45RO^+$). The PBMC *ex vivo* response (20 hr stimulation) to E:C or PPDb consisted of ~56-59% T effector cells ($CCR7^-$, $CD62L^{lo}$, $CD45RO^-$) and ~37-42% Tem cells. Only 3-4 % of the *ex vivo* response consisted of Tcm cells. In response to E:C, expression (MFI) of CD62L differed significantly among Tcm (189 ± 47), Tem (68 ± 14) and T effector (13 ± 2) cells. CD44 expression on Tcm (71 ± 8) in E:C stimulated cultures exceeded that of Tem (25 ± 3) and T effector (27 ± 3) cells. Similar responses to PPDb were observed. These findings confirm that the 14 day cultured ELISPOT assay to *M. bovis* specific antigens is primarily a measure of Tcm responses by cattle to *M. bovis* infection; whereas, *ex vivo* responses are primarily a measure of T effector responses.

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Preterm piglets are a clinically relevant model of pediatric GI disease
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The goal of our research is to establish how nutritional support, enteral versus parenteral, affects gut function and susceptibility to disease in early development. We and others have used the neonatal pig to establish unique models of clinically-relevant problems in pediatric gastroenterology, especially necrotizing enterocolitis (NEC). We are using the premature pig model to establish the critical elements of pathogenesis and strategies for prevention of NEC. Among the established neonatal animal models of NEC, piglets and rodents have dominated the field. Advantages for rodents include the low cost, rapid postnatal development, and capacity for genetic modification, but their size and differences in gastrointestinal tract (GIT) development from humans makes nutritional studies more difficult. Piglets, however, have more anatomic, physiologic, and immunologic similarities to humans, and their size allows for ease of surgical procedures, blood sampling, and dietary manipulation. The similarities in GIT function and development of preterm pigs to those of preterm human neonates is also critical in studying diseases of prematurity such as NEC. Our previous studies using piglets established that prematurity and microbial colonization are necessary elements in the pathogenesis of NEC. More recently we have shown that total parenteral nutrition (TPN) support prior to introduction of formula feeding

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increases the incidence of NEC. We also demonstrated that malabsorption of carbohydrates, namely maltodextrins, in infant formula can trigger bacterial overgrowth and promote the onset of NEC. Current studies are aimed at establishing the optimal lipid and carbohydrate composition of infant formula that minimizes or prevents NEC. The presentation will review recent findings and highlight the significance of the neonatal pig as a model for human infant NEC.

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The Pig as a Model for the Study of Adipose Tissue Dysfunction in Obesity.
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Obesity is a major health problem in the United States and many industrialized countries. Current evidence shows that prenatal factors play a major role in the postnatal development of obesity. These prenatal or maternal factors often induce subtle epigenetic changes on genes that regulate critical metabolic control points, leading to obesity development. Most of the studies on prenatal programming of obesity have been based on the use of rodent models. However, because of vast differences in developmental pattern and nutritional requirements between humans and rodents, rodents do not offer a good model for the study of adipose tissue development in humans. The pig is has similar nutritional requirements to humans and equally has substantial adipose tissue at birth. Overconsumption of calories by gestating sows leads to increased adiposity as indicated by increased backfat thickness. Maternal overnutrition also leads to offspring with elevated expression of adipose tissue genes that increase adipocyte differentiation. In addition, maternal consumption of excess calories also leads to a higher insulin concentration in offspring at 3 months of age than animals from dams that were fed normal energy diet. It was also found that being born small for gestational age increased preadipocyte proliferation rate compared to animals born appropriate or large for gestational age, and this may explain the increased obesity in very light weight pigs. Also our work on the regulation of adipose tissue inflammation shows that adipocyte-derived inflammatory cytokines may contribute to the impaired growth of pigs during immune challenge. Therefore, the use of the pig model will allow rapid advances in the understanding of the impact of maternal factors on adipose tissue development and the contribution of local inflammation in adipose tissue to suboptimal growth.

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Nanoparticle based inactivated adjuvanted porcine reproductive and respiratory syndrome virus vaccine elicits superior cross protective immunity
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Porcine reproductive and respiratory syndrome (PRRS) is an economically important disease of pigs, caused by PRRS virus (PRRSV), incurring estimated $664 million losses annually to the US pork industry. Routinely used modified live PRRSV vaccine is implicated in transmission of mutated vaccine virus to susceptible pigs. Unfortunately, currently available killed PRRSV vaccine elicits poor immunity. Therefore, our goal was to develop a safe and protective killed PRRSV vaccine. We adapted two strategies: firstly, entrapped the killed PRRSV vaccine in PLGA [poly(lactide co- glycolide)] nanoparticles (Nano-PRRSV vaccine); and secondly, co-administered the vaccine with a potent adjuvant, *Mycobacterium tuberculosis* whole cell lysate (*Mtb* WCL), that we identified earlier. To analyze the cross-protective efficacy of the vaccine, pigs were co-administered with PRRSV vaccine and *M tb* WCL, either entrapped (Nano-PRRSV vaccine) or unentrapped in nanoparticles, and then challenged with a virulent heterologous PRRSV. Our results indicated that, Nano-PRRSV vaccine co-administered with unentrapped *Mtb* WCL elicited superior cross-protective immunity, indicated by the following immune correlates of protection, both in the lungs and circulation: (1) increased levels of PRRSV specific IgG and IgA antibody titers with enhanced avidity of antibodies in the lungs; (2) upregulated PRRSV specific neutralizing antibody titers; (3) complete clearance of viremia and replicating PRRSV from the lungs; and (4) enhanced frequency of IFN-γ secreting cells in the lungs. In conclusion, our study for the first time demonstrated that adjuvanted Nano-PRRSV vaccine elicits cross-protective immunity against PRRS in pigs. This project was supported by National Pork Board, USDA PRRSV CAP2, and OARDC to RJG.

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H9e peptide hydrogel: a novel adjuvant for PRRS modified live virus vaccine
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Porcine reproductive and respiratory syndrome virus (PRRSV) is well known for its genetic variation and ability to change constantly, avoiding host defenses and establishing long-term infections. Although current vaccines can confer protection against homologous reinfection, their efficacy against heterologous infection is questionable. The objective of this study was to determine whether peptide hydrogel H9e can be used as an adjuvant for PRRS modified live virus (MLV) vaccine and its immunological mechanisms of adjuvanticity. Fifty-five pigs (3-weeks old) were divided into 11 groups (5 pigs/group) including PBS control groups, pigs vaccinated with Ingelvac PRRS MLV vaccine only, and pigs vaccinated with Ingelvac PRRS MLV adjuvanted with hydrogels H9e, A1/2, or Gel 01. Pigs were challenged with PRRSV VR-2332 or MN184A on day 28 post vaccination. Blood samples were collected weekly after vaccination and blood, lung, and lymph node samples were collected at necropsy (10 days post challenge). It was found that pigs vaccinated with H9e adjuvanted PRRS MLV had a higher and longer viremia than control and pigs that received other adjuvanted vaccines. Pigs vaccinated with H9e-PRRS MLV also developed PRRSV-specific antibodies earlier and had a higher titer of neutralizing antibodies than that from pigs in other groups. More importantly, pigs vaccinated with H9e-PRRS MLV had improved protection against both challenge strains of PRRSV with reduced viremia, lung pathology and robust Th1 type of immune responses over those of pigs vaccinated with A1/2-, Gel 01-adjuvanted vaccines or PRRS MLV alone. Although pigs vaccinated with H9e- or Gel 01-adjuvanted vaccines all had lower frequency of T-regulatory cells at the end of this study, only pigs vaccinated with H9e-PRRS MLV had higher frequency of Th/memory cells in tracheobronchial lymph nodes, lung mononuclear cells, and peripheral blood, increased blood concentration of IFN-γ, and reduced level of IL-10 in the blood. Taken together, our studies suggest that peptide hydrogel H9e is a novel adjuvant that can enhance vaccine efficacy by modulating host humoral and cellular immune responses when it is formulated with PRRS MLV vaccine.

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Moving Swine Immunology Forward Through Molecular and Vaccine Technology

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The American Association of Veterinary Immunologists (AAVI) has selected Dr. Michael P. Murtaugh as their 2012 Distinguished Veterinary Immunologist. Dr. Murtaugh is a Professor in the Department of Veterinary & Biomedical Sciences, College of Veterinary Medicine (CVM) at the University of Minnesota. He received his bachelor's degree from University of Notre Dame and his Ph.D. from Ohio State University. He has 184 refereed journal publications, 65 books/book chapters/reviews/invited articles, and 3 patents. Dr. Murtaugh's CV clearly affirms his impact on veterinary immunology research. His work is focused on molecular mechanisms of host defense against infection, with a major emphasis on swine responses to porcine reproductive and respiratory syndrome virus (PRRSV) infection and more recently gut mucosal immune responses to *Salmonella* infection. Dr. Murtaugh's molecular skills were critical to his work in protein engineering and expression. His was some of the earliest work to clone and express swine cytokines and develop anti-cytokine antibodies for quantitation of expression and function; this technology was transferred to numerous companies to develop these reagents for swine immune studies. Over his career Dr. Murtaugh pursued vaccine improvement for viral pathogens of swine; he applied his extensive expertise to explore novel immunologic approaches using different vectors, adjuvants and combinations of target antigens. His research probed disease mechanisms and viral pathogenesis; with colleagues he detailed the phylogeny of PRRSV including the recent high pathogenicity Chinese PRRSV isolates, and explored alternate controls to prevent replication of infectious viruses. Dr. Murtaugh's publications are important references for the field, his reviews are highly cited and his editorial efforts well respected. For AAVI he served as a proactive board member. His most important research contributions have been through fundamental and applied research into molecular mechanisms of disease resistance in swine, training of graduate students and postdoctoral scientists, and service to the profession and to the national and international research community.

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Development of a mouse model for delineating protective immune response(s) specific for epizootic bovine abortion

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Epizootic bovine abortion (EBA) remains a significant cause of late-term abortion in CA, NV and OR beef cattle. A novel deltaproteobacterium (aoEBA) is the causative agent. Adaptive immunity to aoEBA develops following infection as animals are resistant to experimental challenge during a subsequent pregnancy; the basis for this immunity is unknown. The time and expense of conducting EBA experiments in pregnant cattle was the driving force behind development of a mouse model. Immunocompetent mice are resistant to infection with aoEBA while immunodeficient mice develop a chronic wasting disease approximately 60 days post challenge. Thus an adoptive transfer-of-immunity model is well suited to investigate the basis of immunologic basis of protection.

Immunocompetent BALB/c donor mice received three immunizations with aoEBA or placebo prior to spleen cell transfer. Twenty-six BALB/c RAG-1 recipient mice were divided into groups of four and infected with aoEBA; the infection was allowed to proceed for 30 days prior to cell transfer to allow for adequate bacterial expansion. These infected recipient RAG-1 mice received either 1e7 aoEBA-immunized spleen cells, 1e7 1x-PBS immunized cells, or PBS (challenge controls). Recipient mice were euthanized 7 and 14 days post-transfer (dPT). On average, mice receiving aoEBA-immunized cells had significantly lower pathogen loads in necropsy tissues as compared to the sham immunized recipients ($p<0.005$) or challenge controls ($p<0.005$). Mice receiving aoEBA-immunized cells had aoEBA-specific antibody at 14d PT while other mice did not. T and B-lymphocytes were identified at both 7 and 14d PT in all infected mice that had received spleen cell transfers. While percentages of T and B cells were similar in both groups of recipient mice at 7d PT. Though not significant, mice receiving cell transfers from immunized donors had greater numbers of T cells at 14 d PT as compared to mice receiving cells from mock-immunized donors.

The adoptive transfer mouse model proved to be a robust and sensitive model that will facilitate delineation of the complex immune response(s) to aoEBA and to screen potential vaccine candidates as they become available.

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Use of dermal fibroblasts to predict the innate immune response to bovine mastitis

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Staphylococcus aureus is a major pathogen associated with contagious mastitis in dairy cows. Individual responses to this pathogen exhibit a range of severity and duration. The development of an in vitro model that is predictive of an animal's immune response following an intramammary challenge will facilitate more detailed studies into the molecular basis of this variation.

Fibroblasts were isolated from skin samples collected from 53 early-lactation cows and cryopreserved for subsequent experimentation. Cells were revived and challenged with 200ng/ml of Pam2CSK4 (a synthetic TLR2-TLR6 agonist) and their production of IL-8 was used to sequentially rank the animals. Once ranked, five animals from the bottom 20% (low responders; LR) and five animals from the top 20% (high responders; HR) were selected for an intramammary *S. aureus* challenge. Milk somatic cell count (SCC), as determined by DCC (DeLaval), IL-8 and BSA, as determined by ELISA, and bacterial cfu were measured over six weeks following challenge in the right hind-quarter of each cow.

S. aureus was recovered from all ten challenged quarters at 24 h post infection. The mean bacterial cfu was not significantly different between the LR and HR animals throughout the experiment and only one LR animal cleared the infection. However, the mean SCC in HR milk was approximately 1.6 times higher ($P<0.05$) than that of LR milk over the duration of the experiment. Increased ($P<0.05$) levels of IL-8 in milk were observed in both groups from 24 to 96 h post-challenge, but HR milk contained 1.9 times more IL-8 in comparison to LR milk during this period. Milk BSA concentrations rose acutely in both LR and HR animals. At 72 h post-infection, HR milk BSA peaked at a higher ($P<0.05$) level as compared to LR milk BSA.

The HR animals mounted a stronger immune response to the bacteria resulting in a sustained, higher milk SCC and greater tissue damage than LR animals, but this did not aid in clearing the infection. The observed variation in the immune response between these two groups was predicted by the in vitro model of dermal fibroblasts and warrants their use in further investigation of the molecular causes of the variable responses.

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The potential contribution of epigenetic modifications to the animal-specific responses of dermal fibroblasts to LPS.

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Purpose: Substantial variation exists between cows in the ability of their fibroblasts to produce IL-8 in response to LPS in vitro, and there is evidence in support of an association with in vivo responsiveness to LPS or E. coli infection. This study focused on determining potential epigenetic factors controlling for the differential response of bovine dermal fibroblasts to LPS. Methods: Dermal fibroblast cultures were established from 15 heifers and challenged with LPS for 36 h in the presence or absence or DNA demethylating (5-aza-2'-deoxycytidine) and hyper-acetylating (trichostatin A) agents (AZA-TSA). Response to LPS was determined by ELISA quantification of IL-8 production in media. Gene expression following LPS exposure (with or without AZA-TSA) was analyzed using the Affymetrix bovine genome array. Finally, to determine the potential role of DNA methylation on the differential response between animals to LPS, genome wide DNA methylation profiles were generated via methylated-CpG island recovery assay (MIRA-Seq) on DNA from 3 low- and 3 high responding cultures. Results: A 4.5 fold difference in the production of IL-8 was found between the 4 highest (HR) and 4 lowest (LR) ranked cultures. Preconditioning the cultures with AZA-TSA potentiated the IL-8 response to LPS and eliminated the difference between HR and LR cultures. Microarray- and subsequent RT-PCR-determined expression of IL-8, IL-6, TNF-a, SAA, CCL20, and CXCL2, showed marked increases following LPS exposure under AZA-TSA treatment conditions. MIRA-Seq analysis highlighted areas of DNA in which methylation may play a role in the responsiveness of dermal fibroblasts to LPS. Conclusion: The reduction in differential response of LR and HR fibroblasts to LPS due to AZA-TSA suggests a role for epigenetic modification in contributing to between-animal variation in the immune response.

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Interleukin-8 receptor expression in bovine mammary tissue.

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Mastitis is an ongoing issue in the dairy industry which results from a wide variety of immune related responses in the mammary gland. Many of these responses are mediated by interleukin-8 (IL-8) and its receptors CXCR1 and CXCR2. Exposure of the mammary gland to bacteria causes the release of IL-8. Subsequent binding of IL-8 to its receptors induces responses related to host cell survival, migration and chemokine/cytokine production. These receptors are typically regarded as being present on immune cells with an emphasis on neutrophils. However, our lab has discovered both CXCR1 and CXCR2 receptors being expressed natively in the bovine mammary gland. We hypothesize that each receptor will be expressed on distinctive subsets of mammary gland cells. To evaluate this we used dual immunofluorescence on mammary tissue sections from 4 dairy cows in either mid or late stages of lactation, e.g. 59-99 days post-partum or >250 days post-partum, respectively. Preliminary evidence demonstrates CXCR1 and CXCR2 have different patterns of expression. CXCR1 is expressed by epithelial cells and neutrophils. Stage of lactation appears to influence the intensity of staining present for CXCR1, with higher levels of staining and presumably receptor expression being greater in later stages of lactation. Whereas CXCR2 is present on a subset of epithelial cells but mostly by cells with dendritic-type projections that could represent dendritic cells, γδ T-cells, or another cell type. These results indicate that multiple cell types in the mammary gland express receptors for IL-8 and are capable of responding to IL-8 released in the environment. This response may also vary with stage of lactation. Greater knowledge of which cell populations express these receptors and their role in the mammary gland can create novel targets for treatment of mastitis.

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Lipid metabolism by bovine mammary endothelial cells during *Streptococcus uberis* mastitis

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Incidence and severity of *Streptococcus uberis* mastitis is greatest during the periparturient period and prolonged inflammation can result in severe tissue damage and significant milk production losses. Little is known regarding the pathogenesis of *S. uberis* mastitis and the involvement of surrounding tissues and cells. In other inflammatory models, such as atherosclerosis, vascular endothelial cells play a role in regulating the magnitude and duration of inflammation by evoking the production of eicosanoids. Eicosanoids have demonstrated regulatory properties, such as altering vascular permeability and controlling leukocyte infiltration, leading to either enhancement or resolution of inflammation, depending on the environment established by the eicosanoids. The objective of this study was to determine the expression profile of eicosanoids and assess inflammatory responses of endothelial cells following in vitro challenge with *S. uberis* mastitis. Bovine mammary endothelial cells (BMEC) were indirectly co-cultured with *S. uberis* and temporal changes in eicosanoid biosynthesis and pro-inflammatory gene expression were determined. Exposure of BMEC to *S. uberis* significantly increased cyclooxygenase 2 gene expression that coincided with increases in prostaglandin and thromboxane biosynthesis in *S. uberis*-infected tissues. An increase in 15-lipooxygenase was also demonstrated in BMEC following exposure to *S. uberis*. The pro-inflammatory phenotype of BMEC also was enhanced as indicated by significant increases in the gene expression of adhesion molecules and chemotactic cytokines. Additional studies are currently underway to determine which eicosanoids are responsible for both the onset and resolution of BMEC inflammatory responses following *S. uberis* exposure. A better understanding of important host-pathogen interactions that control the severity and duration of *S. uberis* mastitis may lead to improved prevention and control strategies.

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Increased linoleic acid in post-partum bovine monocytes is associated with proinflammatory phenotype.

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Post-partum infectious and metabolic diseases cause most of the disease-related losses to the US dairy industry. Despite numerous studies associating post-partum disease with increased pre-partum plasma non-esterified fatty acids, the mechanisms by which lipid metabolism influence disease remain elusive. A possible mechanism by which plasma fatty acids lead to disease is by changing the fatty acid composition of circulating immune cells. Indeed, we have observed increased linoleic acid content in the peripheral blood mononuclear cells of post-partum cows. In order to investigate this phenomenon, post-partum bovine mononuclear cell populations were modeled in vitro using a macrophage cell line cultured in the presence of increasing doses of linoleic acid. The objective of this study was to identify how the inflammatory response of macrophages was affected by increased linoleic acid content. Fatty acid methyl ester concentration in cells was measured by GC/MS, inflammatory gene and protein expression measured by qPCR and Western blot, and eicosanoid biosynthesis by LC/MS. Results demonstrated

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that proinflammatory gene and protein expression were increased in macrophages which contained the same linoleic acid content as those isolated from peri-partum cows. Changes in cyclooxygenase and lipoxygenase expression and activity indicate that increased eicosanoid biosynthesis from linoleic acid may contribute to increased inflammatory gene and protein expression. A better understanding of the molecular biology of eicosanoid biosynthesis and inflammatory responses may lead to novel interventions that reduce inflammatory-based pathologies. Dietary modification or supplementation, and novel non-steroidal anti-inflammatory drugs, could reduce the prevalence and severity of post-partum bovine disease.

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Age-related susceptibility to R equi infection in foals

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Rhodococcus equi (R. equi) pneumonia represents a significant economic impact to the horse industry worldwide and it is of great concern to horse-breeding farms. While adult horses are resistant to R. equi, foals exhibit a distinct age-related susceptibility to this infection. However, the reasons for this unique susceptibility remain unknown. While opsonization by specific antibodies and killing of R. equi by IFN-γ activated macrophages and cytotoxic T cells play an important role in defense against R. equi in adult horses, the absence of specific antibodies and reduced cell-mediated immune mechanisms in foals likely renders them susceptible to infection with R. equi. To test this hypothesis, we challenged neonatal (<1 week of age, younger (3 weeks of age) and older (6 weeks of age) foals with virulent R. equi. We then determined the relationship between their susceptibility to infection and the presence of R. equi-specific antibodies and R. equi-inducible Th1 cytokine expression. The 6 weeks old foals were found to be highly resistant to R. equi infection when compared to the younger foals. While foals at 3 weeks of age exhibited signs of bacterial colonization in their lungs, there were no pathogenic lesions apparent at necropsy. The neonates were highly susceptible to infection and presented with extensive lung pathology and high numbers of bacteria in the lungs. Thus, foal age was inversely correlated with both the pneumonia score and the bacterial burdens in the lung. There was also an age-related increase in Th1 cytokine expression in the foals such that the oldest foals showed the greatest IFN-γ responses and the youngest foals the least. By contrast, all groups of foals had similar low levels of R. equi-specific antibodies at the time of challenge and similar antibody responses post infection. In conclusion, the age-related susceptibility of young foals to R. equi infection is associated with reduced Th1 immune function at the time of challenge.

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Evaluation of a live attentuated vaccine for Johne's disease

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Purpose: Initial studies with a mutant strain of M. a. paratuberculosis (Map) with a deletion in relA, a global regulator, demonstrated that vaccination of goats with the mutant limited the capacity of wild type Map to establish an infection. The objective of the present study was to extend the studies an show comparable results could be obtained in calves. PCR and bacterial culture revealed relA was immune eliminated and Methods: 6 day old calves were used in the study 3 vaccinated with relA and 3 inoculated with wild type Map. The calves were challenged with wild type Map 1 month later and necropsied 3 months post challenge.
Results: Ex vivo analysis of the proliferative response of PBMC to challenge with live Map showed calves developed an immune response to Map as detected by flow cytometry. Both CD4 and CD8 T cells proliferated in response to Ag stimulation. QRT-PCR showed gene expression was up-regulated for IFN-γ, IL-17, IL-22, and granulysin. PCR and bacterial culture showed ΔrelA was immune eliminated. Bacterial load was significantly reduced in the calves vaccinated with ΔrelA in comparison with calves inoculated with Map.
Conclusions: The findings show that further studies are warranted to test the ΔrelA mutant for vaccine efficacy under field conditions.

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Tumor necrosis factor (TNF)-α diminishes the ability of bovine macrophage to cleave extracellular traps formed in response to *M. haemolytica*

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Bovine respiratory disease (BRD) can lead to a pleuropneumonia that is characterized by intense inflammation, extensive neutrophil infiltration, fibrin and DNA deposition, and consolidation of the lungs. Extracellular DNA has been observed within the airways of cattle with BRD. One possible source of this DNA is from leukocytes that release DNA studded with antimicrobial proteins to form a web-like structure refereed to as extracellular traps. Previous research has shown that bovine neutrophils and macrophages produce extracellular traps (ETs) in response to *Mannheimia haemolytica* and its leukotoxin (LKT) in vitro. Other studies have confirmed that macrophages utilize DNase II, within lysozomes, to degrade apoptotic cell corpses and ejected erythroblast nuclei. These studies also demonstrate that DNase II activity is reduced in the presence of tumor necrosis factor (TNF)-α. Because macrophages are critical for host defense and regulation of inflammation in the lungs, we examined whether they could digest ET and if that activity influenced by TNF-α. Here, we demonstrate that bovine macrophages are able to reduce extracellular DNA produced by activated neutrophils and macrophages in a time-dependent manner. The addition of DNase II inhibitors diminished extracellular trap degradation by bovine macrophages. We also observed an inability to degrade extracellular traps when bovine macrophages were co-incubated with TNF-α. Co-incubation of bovine macrophages with TNF-α and neutrophil ET lead to a reduction in DNase II as observed by Western blot. These data indicate that TNF-α production during *M. haemolytica* infection could lead to a diminished ability of macrophages to degrade neutrophil and macrophage ETs during infection.

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Induction of osteopontin expression in bovine mammary endothelial cells

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The onset of lactation may lead to oxidant stress and contribute to the increased incidence and severity of mastitis during the periparturient period. Osteopontin expression is upregulated during inflammation, but its role in the pathogenesis of mastitis is unknown. The specific functions of osteopontin during disease states include mediating chemotaxis as well as the survival of cells such as macrophages and T cells. Mammary endothelial cells play a critical role in early inflammatory events and are thought to be a major target of oxidative stress during inflammation. Therefore, the objective of this study was to determine the expression of osteopontin by bovine mammary endothelial cells during conditions of inflammation and oxidant stress. Mammary tissues were collected to measure gene and protein expression of osteopontin under inflammatory conditions, while isolated bovine mammary endothelial cells were utilized to measure osteopontin in vitro under conditions of oxidative stress. Coliform-infected bovine mammary artery tissue had significantly increased osteopontin gene expression when compared to uninfected tissue as determined by qPCR. Osteopontin protein expression by endothelial cells was verified in sections of the mammary pudendal artery and parenchymal tissue by immunohistochemistry. Bovine endothelial cells cultured under conditions of oxidative stress expressed significantly more osteopontin when compared to control cultures. This is the first study to document osteopontin production by bovine mammary endothelial cells and the results suggest that the production of osteopontin could be increased during the periparturient period as a result of oxidant stress. Future studies that elucidate osteopontin's role in the pathogenesis of bovine mastitis may lead to the development of targeted therapies that modulate osteopontin expression during the periparturient period.

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Genetic variation in CXCR1 amino acid expression significantly tied to clearance of Streptococcus uberis in an intramammary challenge model

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Purpose:Mastitis, an inflammation of the mammary gland, continues to plague the dairy industry. Novel preventive and therapeutic strategies are greatly needed to help a dairy cow resist and overcome infection. Towards this end our lab has been examining the role of CXCR1 in the mammary gland and mastitis resistance. Recent research has indicated that CXCR1 has amino acid changes at positions 365, 621, 735, 980, and 995 that result in three combinations: VWHKH, VWHRR, and AWQRR. We hypothesize that these amino acid changes influence the ability of a cow to resist infection.
Methods:To test this, Holstein dairy cows (n=40) were challenged intramammarily with Streptococcus uberis within three days of calving.
Results:All cows (100%) with the VWHRR allele (n=5) required antibiotic therapy within 4.4±0.9 days of challenge and exhibited significantly greater bacterial counts, milk and mammary scores, as well as milk leukocyte counts compared to those expressing only one copy of this allele ($p<0.0001$). In contrast, only 2 of 6 cows (33%) with one VHWRR and one VWHKH allele required antibiotic therapy within 4.6±1.4 days of challenge. This group also had the lowest concentrations of S. uberis present in the gland ($p<0.0001$), the second lowest increase in mammary leukocyte counts ($p<0.0001$), and similar milk and mammary scores compared to all other groups except those homozygous for the VWHRR allele. This preliminary evidence suggests the immune response of the VHWRR*VWHKH group was more effective in eliminating a direct bacterial challenge. The remaining three genetic groups demonstrated responses intermediate between those aforementioned.
Conclusions:The ability of the genetic background in CXCR1 to significantly influence the degree of bacterial clearance without the need for antibiotic therapy supports the concept that this receptor is critical for disease resistance. This knowledge, coupled with the discovery of local populations expressing CXCR1 highlights the need to better understand the role of this ligand-receptor complex and how it enables a cow to better resist infection.

RESPIRATORY DISEASES

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Comparing Influenza A virus isolation from oral fluid and nasal swabs in IAV inoculated pigs

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Purpose: Compare the rate of virus isolation of IAV in nasal swabs and pen-based oral fluid from experimentally inoculated swine over time. Methods: This study was performed using 82 PRRSV-, IAV- and *Mycoplasma hyopneumoniae*- negative piglets. A subgroup (n = 28) was vaccinated twice with a commercial multivalent vaccine (FluSure XP™, Pfizer Animal Health). All pigs were randomly assigned to one of 3 treatment groups: negative control, A/Swine/OH/511445/2007 γ H1N1 inoculated, A/Swine/Illinois/02907/2009 Cluster IV H3N2 inoculated. Inoculated pigs received 2ml of virus inoculum ($10^{6.5}TCID_{50}$/ml) intratracheally. Pen-based oral fluid (OF) samples were collected daily on DPI 0-16. Individual nasal swabs (NS) were collected daily on DPI 0-6, then DPI 8, 10, 12, 14, 16. Samples were stored at -80ºC, then randomized and submitted for IAV detection by virus isolation (VI) at the Iowa State University Veterinary Diagnostic Laboratory. Proc GLIMMIX (SAS® 9.2, SAS Institute Inc., Cary NC) was used to analyze data as well as compare sensitivities and specificities of OF and NS in the mixed model. To compare OF and NS results, a pen was classified NS "positive" if any pig in the pen was NS VI positive. Results: Although NS were only determined more sensitive for the H1N1 virus, numerically there were more NS samples submitted than OF. Differences were noted between the number of VI positive pens (NS vs OF) detected for each serotype. by DPI. Nasal swab and OF detection was influenced by vaccination status and DPI. One false positive VI was reported in a NS sample. In unvaccinated pigs, there was no difference in the duration of detection by VI between NS and OF (DPI 6) regardless of serotype. Detection of IAV by VI on both OF and NS was inhibited by vaccination. Conclusion: pen-based OF is a valid and useful sample type for VI in unvaccinated pigs for at least 6 days post infection.The study was supported in part by Pork Checkoff funds distributed through the National Pork Board (#09-193).

RESPIRATORY DISEASES

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Comparing Influenza A virus detection in oral fluid and nasal swabs by a rapid antigen detection kit in IAV inoculated pigs

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Purpose: Compare the detection of IAV in nasal swabs (NS) and pen-based oral fluid (OF) from experimentally inoculated swine over time using the VetScan® Rapid Test. Methods: The study was performed using 82 PRRSV-, IAV- and *Mycoplasma hyopneumonia*- negative piglets. A subset (n = 28) was vaccinated twice with a commercial multivalent vaccine (FluSure XP™, Pfizer Animal Health). All pigs were randomly assigned to one of 3 treatment groups: negative control, A/Swine/OH/511445/2007 γ H1N1 inoculated, A/Swine/Illinois/02907/2009 Cluster IV H3N2 inoculated. Inoculated pigs received 2ml of virus inoculum ($10^{6.5}TCID_{50}$/ml) intratracheally. Pen-based OF samples were collected daily DPI 0-16. Individual NS were collected daily DPI 0-6, then DPI 8, 10, 12, 14, 16. Samples were frozen at -80ºC, then randomized and tested for IAV using a 15 minute antigen detection assay (VetScan® Rapid Test, Abaxis Inc.). Only samples collected on DPIs 0 to 10 were tested. Proc GLIMMIX (SAS® 9.2, SAS Institute Inc., Cary NC) was used to analyze data in the mixed model. MedCalc® v 12.2.1 (2012 bvba) was used to calculate ROC curves and AUC. Results: There were no differences in detection between serotypes, therefore results were summarized by sample matrix (OF or NS). No false positives were observed with the Rapid Test. There were minimal differences in rate and duration of detection between NS and OF in unvaccinated pigs by either serotype. Sensitivity of IAV detection in OF DPI 0-5 improved if the assay was read at 30 rather than 15 minutes (AUC= 0.752 vs. AUC = 0.701) and was equivalent to NS ($p = 0.74$). Detection of IAV in OF was inhibited by vaccination. Conclusion: The VetScan® Rapid Test could be a useful pen-side test for the detection of IAV antigen in swine during acute infection using either OF or NS. The study was supported by Pork Checkoff funds through the National Pork Board (#09-193). VetScan® AIV Rapid Test kits were kindly provided by Abaxis, Inc.

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Comparing Influenza A virus detection in oral fluid and nasal swabs by RT-PCR in IAV inoculated pigs

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Purpose: Compare the rate of detection of IAV by RT-PCR in nasal swabs vs. pen-based oral fluid from experimentally inoculated swine over time. Methods: This study was performed using 82 PRRSV-, IAV- and Mycoplasma hyopneumonia- negative piglets. A subset (n = 28) was vaccinated twice with a commercial multivalent vaccine (FluSure XP™, Pfizer Animal Health) All pigs were randomly assigned to one of 3 treatment groups: negative control, A/Swine/OH/511445/2007 γ H1N1 inoculated, A/Swine/Illinois/02907/2009 Cluster IV H3N2 inoculated. Inoculated pigs received 2ml of virus inoculum ($10^{6.5}TCID_{50}$/ml) intratracheally. Pen-based oral fluid (OF) samples were collected daily on DPI 0-16. Individual nasal swabs (NS) were collected daily on days post inoculation (DPI) 0-6, then DPI 8,10,12,14,16. Samples were held at -80ºC, then randomized and tested for IAV using a matrix screen RT-PCR. Proc GLIMMIX (SAS® 9.2, SAS Institute Inc., Cary NC) was used to analyze data in the mixed model. Results: Overall, RT-PCR was more sensitive in detecting IAV than VI or a Rapid Antigen Test (VetScan®, Abaxis, Inc). False positive PCRs were reported in both OF (n = 1) and NS (n = 3) samples. A pen was classified NS positive if ≥ 1 pig in a pen was NS RT-PCR positive. Using this convention, NS and OF RT-PCR testing results were equivalent through DPI 8, with more OF-positive pens thereafter. Vaccination reduced the duration of detection of IAV in both OF and NS, although RT-PCR positive NS and OF were detected through DPI 14. RT-PCR testing of pen-based OF was equivalent to, or better than, detection using NS at the pen level. Conclusion: Oral fluid is a valid and useful sample type for the detection of IAV by RT-PCR in both unvaccinated and vaccinated pigs for at least 14 days post infection. The study was supported in part by Pork Checkoff funds distributed through the National Pork Board (#09-193).

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Kinetics of influenza A virus (IAV) anti-nucleoprotein antibody (IgM, IgA, IgG) in serum and oral fluid specimens

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Purpose: Previously, a commercial blocking ELISA (MultiS-Screen® Antibody Test Kit, IDEXX Laboratories, Inc.) was shown to effectively detect anti-NP antibodies in swine serum (Ciacci-Zenella et al., 2010) and oral fluid (Panyasing et al, 2012) specimens. Subsequently, we developed antibody isotype-specific indirect NP ELISAs and evaluated the kinetics of anti-IAV antibody (IgM, IgA, and IgG) in serum and oral fluid specimens following IAV infection.
Methods: Eighty-two pigs were placed in 6 treatment groups: (1) unvaccinated, uninoculated controls (UV_{CTRL}); (2) vaccinated, uninoculated controls (V_{CTRL}); (3) unvaccinated, inoculated with H1N1 (UV_{H1}); (4) vaccinated, inoculated with H1N1 (V_{H1}); (5) unvaccinated, inoculated with H3N2 (UV_{H3}); and (6) vaccinated, inoculated with H3N2 (V_{H3}). Serum (n = 819) and oral fluid (n = 510) specimens were tested for IgM, IgA, and IgG responses.
Results: Following inoculation, a serum NP-specific IgM antibody response was only detected in groups UV_{H1} and UV_{H3} at DPI 7, and was undetectable by DPI 21. Likewise, a serum NP-specific IgA antibody response was only detected in UV_{H1} and UV_{H3} on DPI 14. A serum NP-specific IgG antibody response was detected by DPI 14 in groups UV_{H1} and UV_{H3}, achieving its maximum response at DPI 35. The serum IgG response was markedly faster in groups V_{H1} and V_{H3}, reaching a maximum response by DPI 14 and remaining stable through the end of study. An oral fluid NP-specific IgM response was only detected in groups UV_{H1} and UV_{H3} and achieved the maximum response at DPI 8, after which it rapidly declined. An oral fluid NP-specific IgA response was detected in groups UV_{H1}, UV_{H3}, V_{H1}, and V_{H3} on DPI 6. An oral fluid NP-specific IgG responses in groups UV_{H1}, UV_{H3}, V_{H1}, and V_{H3} were detected by DPI 8 and remained stable through DPI 42.
Conclusions: NP-specific IgM, IgA, and IgG antibodies can be detected in serum and oral fluid over times using the indirect ELISAs developed for this study. This suggests the possibility of improved antibody assays for monitoring IAV infections in swine populations using either serum or oral fluid specimens.

RESPIRATORY DISEASES

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Natural killer T cell specific adjuvants potentiates cell-mediated immunity in the pig lungs to an inactivated bivalent swine influenza H1N1 and H3N2 virus vaccine

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Swine influenza is an acute respiratory disease of pigs caused by type A influenza virus and has recently emerged as a major public health concern. Swine influenza viruses (SIV) do not normally infect humans. However, pigs can be infected by both avian and mammalian influenza viruses. Pigs are considered as 'mixing vessels' for SIV because they facilitate reassortment and emergence of new strains of SIV of zoonotic potential. Thus, control of SIV in swine herds is important, but currently used SIV vaccines are not completely efficacious. This study was conducted to determine the efficacy of an UV-inactivated bivalent SIV vaccine comprising of a triple reassortant H1N1 (Sw/OH/24366/07) and H3N2 (Sw/OH/04) viruses, co-administered with two potent adjuvants, phosphatidylinositolmannosides-2(PIM2) and α-Galctosylceramide (α-GalCer), intranasally. Both these adjuvants potentiate the cell-mediated immunity through CD1d-restricted Natural Killer T (NKT) cells. Unvaccinated and vaccinated pigs (with or without adjuvants) were challenged with a heterologous H3N2 SIV and euthanized on post-challenge day 6. Mononuclear cells (MNC) isolated from bronchoalveolar lavage fluid, lung tissue, and thymus were immunostained to determine the phenotype of immune cells by flow cytometry. In the lungs of adjuvanted vaccine received pigs, increase in the frequency of myeloid cells (macrophages and dendritic cells) and rescue in the frequency of total lymphocytes and $CD8^+$Tcells were observed. In the thymus of adjuvanted vaccine group total $CD4^+$ T cells were increased while in unvaccinated SIV challenged pigs the total $CD8\alpha^+$ and $CD4^+CD8^+$ T cells were increased. In the adjuvanted vaccine group higher frequency of IFN-γ secreting γδ T cells, $CD4^+$T cells, and Natural Killer cells (NK cells) was detected in the 48 hr *ex vivo* culture of MNC. Interestingly, in the thymus of non-adjuvanted vaccine group we observed an increased frequency of $CD8^+$ T cells and γδ T cells expressing CD107, a degranulation marker. In conclusion, PIM2 and α-GalCer potentiate the immune responses to bivalent SIV vaccine in pigs. This project was supported by National Pork Board and OARDC, TheOhio State University to MK and RJG.

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Immortalized swine bone marrow epithelial cell line supports influenza virus replication.

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We established spontaneously immortalized epithelial cell line from bone marrow of pigs designated MK-PBMEC. The cells showed typical cuboidal morphology, characteristic of epithelial cells. The cells grew rapidly and expressed epithelial markers such as pancytokeratin, cytokeratin 18 and occludin. The cells expressed sialic acid receptors utilized by avian and mammalian influenza viruses. Avian, human and swine origin influenza viruses replicated in these cells. These cells should be a valuable tool for studying influenza virus pathogenesis and may be substrate for vaccine production.

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Priming by respiratory exposure followed by intramascular boost with RNA particle vaccine in pigs in an influenza challenge model

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I hypothesize that RNA particles (RP) administered intranasally (IN) may be more effective in immunizing growing pigs in presence of maternal antibody than when given intramuscularly due to inducement of antibodies in the respritory tract. In this study, respiratory exposure with RP expressing pandemic H1N1 (A/CA/04/2009, pH1N1) influenza hemagglutinin (HA) protein was administered to pigs followed by intramuscular (IM) RP boosting vaccination, to determine if protection against pH1N1 influenza virus challenge occurs. In the first experiment, an IN RP was shown to prime pigs to subsequent live influenza virus exposure by increasing the hemagglutinin inhibition antibody titer to the virus. However, in a subsequent study, an IN RP prime followed by an IM RP boost did not result in detectable improved protection as compared to an IM only vaccinatin. Thus in the third ongoing study, IM vaccine dose was reduced to a level which does not protect against challenge and thus a determination can be made regarding an IN prime by RP.

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A novel DNA vaccine provided efficient protection to mice against lethal dose of swine influenza virus H1N1

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Swine influenza virus (SIV) is a fast-evolving viral pathogen in pig populations. However, commercial vaccines, based on inactivated viruses, cannot provide protection to heterologous virus infection with induced humoral immunity only and require frequent updates to fight against current isolates. In this study, a B-cell epitope (HA2.30), a quadruplicated Th-cell epitope (NP55), and a quadruplicated CTL epitope (NP147) were fused separately to the C-terminal of VP22c gene from bovine herpesvirus-1 in a modified pcDNA3.1 plasmid. Chitosan was used as an adjuvant to complex and deliver mixed plasmids intranasally. DNA plasmids were also mixed and administered to mice intramuscularly or intranasally. The vaccine stimulated epitope-specific immunity against the two T-cell epitopes in the mice in the intramuscularly vaccinated group before virus challenge. There was no any detectable humoral or cellular immunity in the intranasally vaccinated groups before virus challenge. The intramuscularly vaccinated group showed 100% survival upon a lethal dose of SIV H1N1 challenge. However, chitosan/plasmid and plasmid only vaccinated group only had 20% survival upon virus infection, compared to none in the challenge control group. DNA plasmids via intramuscular administration are effective in mice without adjuvant and provided protection in mice against influenza virus infection.

RESPIRATORY DISEASES

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Migration of the swine influenza virus delta-cluster hemagglutinin N-linked glycosylation site from N142 to N144 results in loss of antibody cross reactivity

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Routine antigenic characterization of swine influenza virus isolates in a high throughput serum neutralization (HTSN) assay found that approximately 20% of isolates were not neutralized by a panel of reference antisera. Genetic analysis revealed that nearly all of the neutralization-resistant isolates possessed a seasonal human-lineage hemagglutinin (δ-cluster). Subsequent sequencing analysis of full length hemagglutinin (HA) identified a conserved N144 present only in neutralization-resistant strains. N144 lies in a predicted N-linked glycosylation consensus sequence N-X-S/T (where X is any amino acid except proline). Interestingly, neutralization-sensitive viruses all had predicted N-linked glycosylation sites at N137 or N142 with threonine (T) occupying position 144 of HA. Consistent with HTSN assay, hemagglutination inhibition (HI) and serum neutralization (SN) assays demonstrated that migration of the potential N-linked glycosylation from N137 or N142 to N144 resulted in a greater than eight-fold decrease in titers. These results were further confirmed in a reverse genetics system where syngenic viruses varying only with predicted N-glycosylation sites at either N142 or N144 exhibited distinct antigenic characteristics as observed in field isolates. Molecular modeling of the hemagglutinin protein containing N142 or N144 in complex with neutralizing antibody suggests that N144-induced potential glycosylation may sterically hinder access of antibody to the hemagglutinin head domain, which allows viruses to escape neutralization. As N-linked glycosylation at these sites have been implicated in genetic and antigenic evolution of human influenza A viruses, we conclude that the relocation of the hemagglutinin N-linked glycosylation site from N142 to N144 render swine influenza virus δ-cluster viruses resistant to antibody-mediated neutralization.

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Inactivation of Swine Influenza Virus with imidazolidinyl urea with retention of hemagglutination activity

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Vaccines for the prevention of disease caused by Swine Influenza Virus (SIV) are inactivated during manufacturing using compounds such as formaldehyde, binary ethyleneimine (BEI), or beta-propiolactone. A new non-toxic compound for inactivation, imidazolidinyl urea (IDU), was tested to determine the ability of IDU to inactivate infectivity of SIV, while retaining biological activity as measured by hemagglutination activity (HA). The purpose of this study was to determine the ability of IDU to stabilize the HA activity of H1N1, H1N2 and H3N2 isolates of SIV using various inactivation conditions and accelerated storage temperatures. The SIV isolates were inactivated with IDU or BEI, and were stored at 2-7°C, 22-25°C, 35-39°C or 40-42°C for extended periods of time. Virus that was not inactivated acted as a control. The HA assays were performed with the homologous virus isolate, and a difference of more than two 2-fold dilutions was considered to indicate a difference in HA between treatment groups. The H1N1 virus was tested for HA activity after storage for up to twenty-one months. The H1N1 isolate was very stable at refrigerated temperatures, and there was no difference in the HA activity of untreated virus, virus inactivated with BEI or IDU when stored at 2-7°C. The H1N1 virus inactivated with IDU retained better HA activity then virus inactivated with BEI when stored at 22-25°C. Overall, inactivation of H1N1 with IDU provided as good or better HA as BEI inactivation. The HA of the H1N2 and H3N2 virus (inactivated with IDU and BEI) was determined for samples stored for up to one year. Inactivation with IDU did not provide better stability for the preparation of the H1N2 isolate used in this study. Inactivation of the H3N2 isolate with IDU provided similar stability of HA compared to BEI inactivation at most temperatures. Inactivation of H3N2 with IDU provided better stability of HA for virus stored at 40-42°C. Inactivation of SIV with IDU while retaining HA activity was demonstrated for several SIV isolates. The IDU represents a novel approach to virus inactivation, stability, and manufacturing for vaccine production. (This work was done as a funded program with Streck Corporation).

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Full genome of swine influenza (H1N1) in pigs using next generation sequencing

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In this study we report the results of sequencing the entire genome of an H1N1 influenza virus directly from nasal swab samples using a single reaction genomic amplification previously described and a high fidelity polymerase as an effort to describe virus diversity across the complete genome of a swine influenza virus.
Nasal swabs were obtained from pigs infected with influenza virus by contact exposure with an infected pig challenged with an H1N1 swine influenza virus (A/Sw/00239/04). Samples were tested by RT-PCR and one sample from each pig testing positive and the virus challenge inoculum were selected for full genome sequencing. cDNA was prepared and sequenced using the 454 genetic sequencing platform.
Full length sequences were obtained for all 8 segments in all the samples analyzed. The gene nucleotide length for swine influenza A/Sw/00239/04 segments 1, 2, 3, 4, 5, 6, 7 and 8 was determined to be 2316, 2314, 2250, 1776, 1563, 1410, 1030, and 851 respectively. For each segment between 169 and 71060 reads were obtained giving enough coverage for each position to assess diversity within and between samples.
The genetic analysis of this study is in progress and results will be presented at the meeting.
The methods used in this study allowed to characterize swine influenza viruses directly from nasal samples which may help avoid viral selection that can occur during virus isolation.
Acknowledgements
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RESPIRATORY DISEASES

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Genome evolution and antigenic variation of canine influenza virus H3N8 in U.S. dogs

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Purpose: Canine influenza virus (CIV) H3N8 has spread rapidly throughout dog populations in the U.S. since it was first identified in Florida racing greyhounds as a canine respiratory agent in 2004. Recent serological and nasal swab data from humane shelter surveillance studies suggest that CIV is being brought into shelters from the non-shelter community and subsequently reintroduced into the outside community when shelter dogs are adopted. As influenza viruses evolve rapidly due to host immune pressure, leading to an accumulation of mutations in the virus' antigenic proteins, and as continuous circulation of CIV in shelters might result in accelerated genetic variability of the virus, we sought to determine the evolution of CIV in the U.S. dogs since emergence in 2004.
Methods: To this end, the full-length hemagglutinin gene of 19 CIV isolates from dogs sampled from Colorado, New York, and South Carolina humane shelters from 2009 to 2012 were sequenced. Both the nucleotide and amino acid sequences were compared to all those published for CIV strains isolated since 2003 and phylogenetic and selection pressure analyses were performed.
Results: Phylogenetic analysis suggests that CIV is diverging into two genetically distinct clades. Using a mixed-effects model for evolution (MEME), five amino acid sites were found to be undergoing episodic selection pressure. Additionally, a total of five amino acid changes were observed in two antigenic sites for CIVs isolated from the New York and South Carolina humane shelters between 2009 and 2011.
Conclusions: The findings reported here support previous studies that suggest CIV is diverging into two genetically distinct clades (Colorado and New York). Although preliminary data suggest that the New York clade age is evolving into a distinct antigenic cluster, controlled experiments are required to determine true extent of antigenic drift occurring within circulating CIV.

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Zoonotic tuberculosis in pastoralists and their livestock in Ethiopia

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The occurrence of human tuberculosis (TB) cases and their main causative strains in remote pastoral zones of Ethiopia are hardly known. In these zones people live in close contact to their livestock, and proportion of human TB cases could be due to infection with cattle strains (Mycobacterium bovis). To investigate the presence of zoonotic transmission of tuberculosis in these zones, study was conducted from March 2008 to February 2010. Specimens from suspected TB patients (sputum and fine needle aspirates of swollen cervical lymph nodes) and cattle, sheep and goats at slaughterhouse were collected and cultured at TB laboratory in two pastoral areas of South-East Ethiopia. Positive cultures were differentiated with spoligotyping. Most human isolates (160/173) were Mycobacterium tuberculosis, but three were M. bovis. Twenty-four M. bovis strains were isolated form cattle and one M. tuberculosis from a camel. Given that M. bovis was isolated from people and one strain was identical to a strain from cattle, and M. tuberculosis from livestock in this study strongly suggests that tuberculosis is transmitted between livestock and humans.

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The impact of gastrointestinal nematode parasitism on the response of calves to viral respiratory vaccination

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Gastrointestinal nematode (GIN) parasitism is a common problem in cattle although the magnitude of infection varies among regions. In addition to direct effects on the host, evidence from other species suggests that helminth parasitism impacts health by impairing the immune response to infectious agents and vaccines. This pilot study tested the hypothesis that GIN parasitism in beef calves modifies the immune response to viral respiratory vaccines. Forty nursing beef calves were randomly assigned to one of two groups. The "low parasite" (LP) group was treated monthly from 2 to 6 months of age with 2 anthelmintics (oral fenbendazole and subcutaneous moxidectin), while the "high parasite" (HP) group was mock treated with oral water and injectable sterile saline. Calves were vaccinated with a subcutaneous modified live virus vaccine containing BHV-1, BVDV1, BVDV2, BRSV, and PI3V at 6 months of age and boosted at 7 months of age. Feces were collected for fecal egg counts (FEC) when calves were 5, 6, and 8 months old. Serum was collected for measurement of serum neutralizing (SN) titers to BVDV1 and BHV-1 when calves were 2, 5, 6 and 8 months old, and calves were weighed. Peripheral blood mononuclear cells were collected when calves were 5, 6, and 8 months old for measurement of BHV-1 and BVDV1 specific interferon gamma (IFN gamma) and interleukin 4 (IL-4) production. The LP calves had lower FEC at 5 and 6 months of age: geometric means (95% CI) at 5 months: 145 (115, 182) epg for HP vs 4.0 (2.0, 8.3) epg for LP, $p < 0.001$; at 6 months: 55 (36, 86) epg for HP vs 1.4 (0.0, 2.0) epg for LP, $p < 0.001$. There was no difference in weights of calves at any time. The HP calves had significantly ($p < 0.05$) lower BHV-1 SN titers at 8 months than LP calves. In LP calves, there was a trend ($p = 0.067$) toward increased production of IL-4 in response to BHV-1 at 8 months, and a trend ($p = 0.067$) toward increased production of IFN gamma in response to BVDV at 5 months. These results support the hypothesis that parasitism impacts the immune response of beef calves to vaccination. Additional studies are required to further elucidate the host-parasite interactions that impact vaccine responsiveness in cattle.

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In vitro and in vivo prostaglandin E2 synthesis in BRSV infection and modulation by COX inhibition

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Infection of the respiratory tract by virus is generally accompanied by virus-induced inflammation. The synthesis of prostaglandins is initiated when the cell membrane is perturbed and releases arachidonic acid from membrane glycerophospholipids. Cyclooxygenase enzymes (primarily COX-1 and 2) initiate production of prostaglandins from the intermediate PGH_2. Prostaglandin E_2 is of particular interest to respiratory disease as

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it effects immune cell function. PGE_2 has been shown to suppress production of Th1 cytokines and to facilitate Th2 cytokine synthesis. Bovine respiratory syncytial virus (BRSV) has been shown to express a strong Th2 bias. We performed experiments to evaluate the role of prostaglandin E_2 in the pathogenesis of bovine respiratory disease caused by BRSV. First we examined the effect of BRSV infection on bovine turbinate cells and on bovine alveolar type 2 cells by microarray. In both cell types BRSV up-regulated expression of prostaglandin synthase compared to uninfected controls. We further evaluated by quantitative RT-PCR the levels of COX-2 expression in cells infected with BRSV compared with cells infected with bovine herpes type 2 (IBR) virus or control and found that BRSV alone enhanced both COX-2 expression and PGE_2 production. Meloxicam, a COX-2 inhibitor, decreased levels of PGE_2 in a dose dependent manner in BRSV infected cell culture. Steers infected with BRSV and *Histophilus somni* similarly showed enhanced PGE_2 production in response to infection, which was modulated by a one time injection of Meloxicam on day 8 post viral infection. These studies demonstrate the role of prostaglandins in initiation and progression of lung inflammation in bovine respiratory disease caused at least in part by BRSV; and support the rationale for the use of non-steroidal anti-inflammatory drug use in treatment.

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Prevalence of viral and bacterial pathogens in nasopharyngeal and pharyngeal recess regions of Holstein calves with and without signs of clinical bovine respiratory disease
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Purpose: The objective of this research was to perform a case-control study of bovine respiratory disease (BRD) in young Holstein bull and heifer calves ranging in age from 35 to 55 d at a large calf ranch in central California to evaluate the association of viral and bacterial pathogens from the nasopharyngeal and pharyngeal recess regions. Methods: A total of 1,004 calves were identified as BRD cases which had scores of 5 or greater based upon the University of Wisconsin calf respiratory scoring system which evaluated rectal temperature, cough, nasal and ocular discharges, and ear position or head tilt. These calves were paired with a similar number of control calves that had minimal signs or no clinical evidence of BRD. Calves were sampled for BRD pathogens prior to antimicrobial treatment. Both mid-pharyngeal and deep-pharyngeal swabs were collected for viral PCR diagnostics that included bovine viral diarrhea virus (BVDV), bovine coronavirus (BCV), bovine respiratory syncytial virus (BRSV), and infectious bovine rhinotracheitis virus (IBR). Another deep pharyngeal swab was collected for aerobic microbiological and mycoplasma culturing. Results: BRD cases had a significant association with serum total protein <5.2 g/dL at 2 d of age compared to controls (OR = 1.42; 95% CI = 1.19, 1.69). Diagnostic results yielded significant associations of cases with these pathogens compared to controls: BCV (OR = 1.29; 95% CI = 0.94, 1.78); BRSV (OR = 2.89; 95% CI = 2.19, 3.82); *Mycoplasma* spp. (OR = 1.29; 95% CI = 1.08, 1.54); *H. somni* (OR = 4.40; 95% CI = 1.48, 13.12); *Mannheimia* spp. (OR = 2.49; 95% CI = 1.95, 3.19); *P. multocida* (OR = 1.95; 95% CI = 1.61, 2.37). All samples were negative for IBR and BVDV. Conclusions: Cases of respiratory disease in young Holstein calves which were identified by a respiratory scoring system had significant associations with some but not all BRD viral and bacterial pathogens. Negative results for BVDV PCR on 2,030 samples indicated that maximum BVDV pathogen prevalence would be less than 0.14% among this population of calves in hutches on this calf ranch. Findings from this study will be used to evaluate genetic relationships of Holstein calves for resistance or susceptibility to BRD.

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Pathogenicity of *Bibersteinia trehalosi* in cattle
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Purpose: *Bibersteinia trehalosi* is a known cause of disease in ruminants worldwide. *B. trehalosi* is typically,associated with pneumonia or septicemia in sheep. Although infection with *B. trehalosi* is rare in cattle, it is a potential agent responsible for cattle respiratory disease. Anecdotal reports of increasing prevalence of *B. trehalosi* in cattle with severe disease have heightened producer and veterinary awareness. The purpose of this study was to determine pathogenicity of different *B. trehalosi* isolates in cattle.
Methods: Thirty-five 2-3 month old calves were inoculated using an intra-tracheal catheter with either: BHI broth control media, 2.5 x 10^9 CFU of leukotoxin negative *B. trehalosi*, leukotoxin positive *B. trehalosi*, *Mannheimia hemolytica*, or a combination of leukotoxin negative *B. trehalosi* and *Mannheimia hemolytica*. Calves were monitored twice a day and humanely euthanized if moribund. All calves were humanely euthanized on day 10 (inoculation on day 1) and lungs examined both grossly and histologically.
Results: Calves inoculated with either Leukotoxin negative *B. trehalosi* or Leukotoxin positive *B. trehalosi* had moderate percentage of consolidated lung tissue with the leukotoxin positive challenged calves having slightly more lung consolidation. *M. hemolytica* inoculated calves had more extensive percentage of lung involvement than either of the *B. trehalosi* isolates alone. Additionally, the mixed inoculation of *M. hemolytica* and leukotoxin negative *B. trehalosi* resulted in very extensive consolidation of lung tissue. Histopathology was consistent with extensive pyogranulomatous bronchointerstitial pneumonia.
Conclusion: Calves inoculated with *B. trehalosi* bacteria developed moderate degree of bronchopneumonia. Leukotoxin positive *B. trehalosi* appeared to induce more severe pathological changes. Additionally, there appeared to be a synergistic effect with a mixed infection of *M. hemolytica* and *B. trehalosi*.

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RNA-Seq based structural re-annotation of BRD bacterial pathogens
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Holistic systems biology approaches can improve our understanding of multifactorial diseases such as bovine respiratory disease (BRD) in cattle. Our long-term goal is to investigate the interactions among the host, microbial pathogens, and the environment to shed light on BRD pathogenesis for identifying potential points of intervention. To enable systems biology analysis, a description of all the functional elements in

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pathogens' genomes is required. In particular, there is need for re-annotation of pathogen genomes prior to systems analysis. Re-annotation entails updating the information pertaining to functional DNA, RNA, and protein in a genome sequence. The focus of the current study is structural re-annotation of three gram-negative BRD bacterial pathogens, Mannheimia haemolytica (PHL213, serotype 1 bovine isolate); Pasteurella multocida (3480, serotype A porcine isolate); and Histophilus somni (2336, bovine isolate). We used RNA-Seq to re-annotate the transcriptomes for these three stains utilizing Illumina platform. The computational challenges associated with the data analysis and interpretation will be discussed. We will also summarize the overall improvements made to the existing structural annotation of these genomes. We identified operon structures, potential novel sRNA, and protein coding regions. For example, P. multocida RNA-Seq generated ~ 17 million reads. Reads were aligned to the genome and analyzed using SAMTOOLS and custom perl scripts to identify expressed regions. Evaluation of intergenic expressed regions identified 53 small RNA. Alternate/mutated start/stop sites were found for 23 genes, and we identified 20 frameshift mutations. We also identified 44 potential novel protein coding regions missed in the current annotation. Biological characterization of these novel elements in the context of infection has the potential to provide new information on BRD pathogenesis.

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Mannheimia haemolytica immunity. Are we there yet?
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Mannheimia haemolytica immunity. Are we there yet? A. W. Confer. Dept. of Veterinary Pathobiology, Center for Veterinary Health Sciences, Oklahoma State University, Stillwater, OK.
Mannheimia haemolytica (formerly *Pasteurella haemolytica* biotype A) is the main cause of severe, often fatal pneumonia in stressed cattle. Numerous laboratories in North America and abroad have studied immunity, pathogenesis, and vaccination related to *M. haemolytica* infections of cattle. Several findings in the 1970s and 1980s were seminal and critically important in our understanding of *M. haemolytica* and bovine respiratory disease. Those key findings include 1) understanding that cattle with high antibodies to *M. haemolytica* upon feedlot entry had less disease (Thomson et al., CJCM, 1975), 2) marked changes occurred in the nasal bacterial flora during stress and viral infection allowing inhalation of the bacterium (Grey & Thomson, CJCM 1971; Frank & Smith, AJVR 1983, Frank et al., AJVR 1986), 3) *M. haemolytica* secretes a ruminant-specific leukotoxin (Shewen & Wilkie, Inf Imm 1982), 4) leukotoxin-neutralizing antibodies are produced and correlate with immunity (Shewen & Wilkie, CJCM 1983; Gentry et al. VII 1985), 5) immunity to leukotoxin and to surface antigens are required for immunity (Shewen & Wilkie, CJVR 1988), and 6) the major surface antigens are proteins, most likely outer membrane proteins (Mosier et al., Inf Imm 1989; Confer et al., AJVR 1986; Confer et al., AJVR 1989). This presentation will review important findings on *M. haemolytica* immunity, discuss what we still need to know, and outline future directions for improved vaccine development. Future directions include greater understanding of important surface immunogens, recombinant protein-based or recombinant protein-augmented vaccines, membrane vesicle vaccines, genetically modified vaccines, and alternative delivery methods.

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DNA shuffling of the GP3 genes of PRRSV produces a chimeric virus with an improved cross-neutralizing ability against a heterologous PRRSV strain
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Purpose: Porcine reproductive and respiratory syndrome virus (PRRSV) is an important swine pathogen. Here we applied the DNA shuffling approaches to molecularly breed the PRRSV GP3 gene, a neutralizing antibodies inducer, in an attempt to improve its heterologous cross-neutralizing ability. Methods: The GP3 genes of six different PRRSV strains were bred by traditional DNA shuffling. Additionally, synthetic DNA shuffling of the GP3 gene was also performed using degenerate oligonucleotides. The shuffled-GP3-libraries were cloned into the backbone of a DNA-launched PRRSV infectious clone pIR-VR2385-CA. Results: Four traditional-shuffled chimerias each representing all 6 parental strains and four other synthetic-shuffled chimeras were successfully rescued. These chimeras displayed similar levels of replication both in vitro and in vivo, compared to the backbone parental virus, indicating that the GP3 shuffling did not impair the replication capability of the chimeras. One chimera GP3TS22 induced significantly higher levels of cross-neutralizing antibodies in pigs against a heterologous PRRSV strain FL-12. Conclusions: DNA shuffling of GP3 gene of PRRSV can improve its heterologous cross-neutralizing ability.

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Characterization of the neutralizing antibody response to PRRSV
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Purpose: Effective host immune countermeasures to novel pathogens is essential to prevent new pandemic diseases. Inability to elucidate key protective elements of the immune response to porcine reproductive and respiratory syndrome virus (PRRSV) has frustrated rational design of vaccines that prevent high consequence PRRSV outbreaks and disease transmission. Our goal is to elucidate mechanisms of protective immunity to prevent PRRS and interdict disease spread in outbreak incidents. Here, we examined the role of neutralizing antibodies directed to glycoprotein 5 (GP5) and membrane (M) protein, the major proteins in the PRRSV envelope. Although viral neutralization epitopes are reported in GP5 and M of type 2 PRRSV, their significance as targets of porcine humoral immunity is not well described.
Methods: We constructed recombinant polypeptides containing ectodomain neutralization epitopes to examine their involvement in porcine antibody neutralization and antiviral immunity.
Results: PRRSV infection elicited ectodomain-specific antibodies, whose titers did not correlate with the neutralizing antibody (NA) response. Ectodomain-specific antibodies from PRRSV-neutralizing serum bound virus but did not neutralize infectivity. Furthermore, immunization of pigs with ectodomain polypeptides raised specific antibodies and provided partial protection without a detectable NA response. Finally the polypeptides did not block infection of porcine macrophages.
Conclusions: These results suggest that the GP5/M ectodomain peptide epitopes are accessible for host antibody recognition, but are not associated with antibody-mediated virus neutralization.

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Development of swine oral fluid based porcine reproductive and respiratory syndrome virus neutralizing assay: a potential diagnostic tool for PRRS herd immunity

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Porcine reproductive and respiratory syndrome (PRRS) is one of the economically important viral diseases of pigs. PRRS virus neutralizing (VN) antibodies are produced following infection or vaccination and are important in providing protection against the disease. To evaluate PRRS VN antibodies we need to collect blood samples, and collecting statistically significant numbers in large commercial farms is not realistic. But in other viral disease models like rhinovirus, HIV, CMV etc., oral fluid samples were shown to possess VN activity. Evaluation of oral fluid samples for PRRS VN titers may provide useful information about collective VN activity mediated by pig innate immune factors (non-specific immunity) and secretary antibodies (specific immunity). We standardized an oral fluid based PRRSV VN assay using samples obtained over a period of three months from swine herds vaccinated with modified live PRRSV vaccine. In our study the VN titers against North American prototype PRRSV strain VR2332 was determined by indirect immunofluorescence assay. Further, to establish a correlation between PRRSV VN titers in serum and oral fluid samples, we obtained both oral fluid and serum samples (five representative sera from each pen) from a total of 104 pens (n=25 pigs/pen) of wean-to-finish pigs, belongs to different commercial swine farms in midwest USA, with the history of PRRSV infection. Our initial results established a low level of correlation between serum and oral fluid PRRS VN titers, and such evaluation is still in progress with more number of field and experimental samples. In conclusion, we are in the process of validating an assay to monitor PRRS VN titers in oral fluid samples, and this tool may be beneficial to assess PRRS herd immunity in vaccinated and/or infected recovered swine herds. This project was supported by USDA-NIFA PRRS CAP2 and OARDC, The Ohio State University to RJG.

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Effect of sample collection material on the detection of PRRSV in oral fluid

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Reports in human diagnostic medicine suggest that the material used to collect oral fluid samples can affect the detection of diagnostic targets.[1,5] The objective of the present study was to determine if the material used to obtain pen-based swine oral fluid specimens affected the results of PRRSV antibody- or nucleic acid-based testing.

Oral fluid samples were collected from 104 pens on 2 commercial swine farms. Three oral fluid samples[2,3,4] were collected in succession from each pen using ropes made either of cotton (C), hemp (H) or nylon (N). To account for the possible effect of collection order, samples were collected from pens in 1 of 3 sampling sequences: C-N-H, N-H-C, or H-C-N. Samples were then assayed for PRRSV antibody[3] and PRRSV RT-PCR. The effect of sampling material and collection order on ELISA sample-to-positive (S/P) ratio was analyzed by ANOVA. Qualitative results of PRRSV RT-PCR testing were analyzed for differences in sampling material and collection order using logistic regression.

Analysis of the results found sampling material affected diagnostic results, but cotton rope provided the best overall diagnostic performance. Therefore, oral fluid samples should be collected using cotton-based materials.

Acknowledgments

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Probability of detecting PRRSV infection using pen-based swine oral fluid specimens as a function of within-pen prevalence

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Introduction Pen-based oral fluid sampling allows for the collection of samples that represent a large number of animals. For interpretation of results, however, performance of oral fluid assays (antibody- and nucleic acid-based) in populations of low disease prevalence must be established. Therefore, the objective of this study was to determine the probability of detecting PRRSV infection in pen-based oral fluid samples from pens of known PRRSV prevalence.

Materials and Methods In one commercial swine barn, 25 pens were randomly assigned to 1 of 5 levels of PRRSV prevalence (0%, 4%, 12%, 20%, or 36%). PRRSV prevalence was established by placing a fixed number (0, 1, 3, 5 or 9) of pigs 14 days post-vaccination with a MLV

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PRRSV vaccine in pens such that the combination of negatives and positives in each pen totaled 25 pigs. In total, 6 oral fluid samples were collected from each of the 25 pens (n = 150).
To confirm individual pig PRRSV status, serum samples from the PRRSV-negative pigs (n = 535) and the PRRSV vaccinated pigs (n = 90) were tested for PRRSV antibodies and PRRSV RNA. The 150 pen-based oral fluid samples were assayed for PRRSV antibody and PRRSV RNA at 6 laboratories.
The overall probability of detecting PRRSV infection in one pen-based oral fluid sample was calculated with logistic regression for both assays using the results from all laboratories.
Results and Conclusions The probability of detecting a PRRSV-positive pen-based oral fluid sample was greater than 90% for both assays, in pens with a within-pen PRRSV prevalence of at least 32%.
Overall, this data supports the use of pen-based oral fluid sampling and testing by either ELISA or PCR for PRRSV surveillance in commercial pig populations.

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

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

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The antiviral activity of *Actinobacillus pleuropneumoniae* against Porcine reproductive and respiratory syndrome virus in the porcine alveolar macrophages

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Respiratory diseases in pigs are caused by the concomitant presence of one or more pathogens. Virus-bacteria mixed infections such as porcine reproductive and respiratory syndrome virus (PRRSV) and *Actinobacillus pleuropneumoniae* (App) could lead to porcine respiratory disease complex. A recent study have demonstrated that the culture supernatant of a strain of App serotype I, inhibits the replication of PRRSV in the newly discovered SJPL permissive monkey cell line but not in MARC-145 cell line. Since the primary target cells of PRRSV in the naturally infected host are the porcine alveolar macrophages (PAM), therefore it would be interesting to determine whether this phenomenon also occurs in those cells. The objective of this study is to demonstrate the antiviral effect of the supernatant of App against PRRSV in primary cultures of PAM and to study the specific mechanisms involved in the viral inhibition. First, the PAM were infected with the PRRSV reference strain IAF-Klop and then treated with the culture supernatant of App. Viral titer, cell survival, cell death, mRNA expression of cytokines and expression level of actin filaments in the cell were measured in the absence or presence of the supernatant of App. In the presence of the App supernatant, one log10 of viral titer decrease, an increase of cell survival and a decrease in cell death were observed in PRRSV infected cells. In addition, actin filaments were disrupted by App supernatant compared to PRRSV infected PAM without App supernatant. The RT-qPCR data shows that there is no induction of type I IFNs (IFN-α and IFN-β) and IFN-γ in the presence of App culture supernatant, but an increase of IL-8 mRNA expression level was observed in co-infected PAM. Thus, we report for the first time the App antiviral effect against PRRSV in PAM. Interestingly, this study suggests that one of the specific mechanisms used by the bacterial supernatant to inhibit PRRSV infection could be via the modulation of the actin filaments pattern, since it was previously shown that PRRSV requires an intact actin cytoskeleton for cell infection.

VECTOR-BORNE AND PARASITIC DISEASES

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Targeted and Random Mutagenesis of *Ehrlichia chaffeensis* for the Identification of Genes Required for *In vivo* Infection

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Ehrlichia chaffeensis is a tick transmitted rickettsial pathogen responsible for the disease, human monocytic ehrlichiosis. Research to elucidate gene function in rickettsial pathogens is limited by the lack of genetic manipulation methods. Mutational analysis was performed targeting to specific and random insertion sites within the bacterium's genome. Targeted mutagenesis at six genomic locations by homologous recombination and mobile group II intron-based methods led to the consistent identification of mutants in two gene coding regions and in one intragenic site; the mutants persisted in culture for up to 8 days. Three independent experiments using Himar1 transposon mutagenesis in *E. chaffeensis* resulted in the identification of mutants; these mutants grew continuously in macrophage and tick cell lines and included several insertions, 9 of which were confirmed by sequence analysis. Six insertions were located within non-coding regions and three were present in the coding regions of three transcriptionally active genes encoding for hypothetical proteins. The intragenic mutations prevented transcription of all three genes. The transposon mutants from one of the experiments containing five different insertions were assessed for their growth in white-tailed deer and acquisition by *Amblyomma americanum* ticks from infected animals. Three of the five mutants with insertions into non-coding regions grew well in deer. Disruption of a differentially expressed gene, Ech_0379, and at an intergenic site located between the genes Ech_0230 and Ech_0231 resulted in the lack of growth of the mutants in deer, which is also further evidenced by their failed acquisition by ticks. This is the first study to evaluate mutagenesis in *E. chaffeensis* and demonstrates that disruption of a specific gene limits the pathogen growth *in vivo*.

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Exploratory spatial data analysis of human Lyme disease cases in Texas between 2000 and 2010

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Lyme disease is a debilitating, tick-borne zoonotic illness caused by the bacterium Borrelia burgdorferi. The disease is suspected to be emerging in several states including Texas due to climate change, however epidemiologic studies are lacking. The goal of this study was to analyze human Lyme disease cases reported to the Texas Department of Health with the objectives to investigate 1) the spatial patterns of Lyme disease and 2) the association between climatic factors and Lyme disease risk in humans in Texas. County level cumulative incidence data were used to calculate the univariate global Moran's I statistics based on rook contiguity spatial weights to assess the presence of spatial autocorrelation. Univariate local indicator of spatial association (LISA) was used to determine location of spatial clusters and outliers. Available data at the zip code level were used to investigate the relationship between climatic variables and Lyme disease incidence using bivariate global Moran's I statistics and spatial regression (spatial lag and error) models. Climatic variables included the10-year average monthly minimum and maximum temperature and relative humidity. Census data were used as population at risk. There were a total of 1,212 cases reported from 138 out of 254 counties in Texas over the period 1/1/2000 to 12/31/2010. Forty percent of all cases were reported from the metropolitan areas of Austin, Houston, and Dallas. The number of cases per year ranged from 29 in 2006 to 276 in 2009. The univariate global Moran's I value was 0.32 ($p>0.001$) indicating an overall positive spatial autocorrelation for Lyme disease incidence in Texas. Univariate LISA revealed that the Western Cross Timbers ecoregion had high-risk while the Low Plains region had low-risk for human Lyme disease. Data from 1,133 cases in 500 zip codes were available for further analyses. Bivariate Moran's I and spatial regression models suggested positive association between Lyme disease incidence and maximum temperature. The exploratory analysis of human Lyme disease cases identified a high-risk area in Central Texas and suggested a climatic trend. Ongoing studies to incorporate tick and dog data will refine these findings.

VECTOR-BORNE AND PARASITIC DISEASES

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Transplacental transmission of a human isolate of *Anaplasma phagocytophilum* in an experimentally infected sheep.

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Anaplasma phagocytophilum (Ap), first identified as a pathogen of ruminants in Europe, has more recently been recognized as an emerging tick-borne pathogen of humans in the U.S. and Europe. Transmission of Ap is mainly by ticks, primarily of the genus *Ixodes*. While mechanical and transplacental transmission have been reported for *A. marginale*, these modes of transmission have not been considered for Ap. However, perinatal transmission of Ap was reported previously in an experimentally infected cow and a naturally-infected human. Recently, we developed a sheep model for studying host/tick/pathogen interactions of the human NY-18 Ap isolate. While sheep were susceptible to infection with this human isolate and served as a source of infection for *I. scapularis* ticks, they did not display clinical signs of disease and the pathogen was not readily demonstrated in stained blood smears. In the course of these Ap/sheep experiments, one sheep unexpectedly gave birth to a lamb 5 weeks after being experimentally infected by inoculation with Ap. The lamb was depressed and was subsequently euthanized 18 hrs after birth. A necropsy was performed, and blood and tissues were collected for microscopic examination and for PCR in order to confirm Ap infection. At necropsy, the stomach contained colostrum, the spleen was moderately enlarged and thickened with conspicuous lymphoid follicles and mesenteric lymph nodes were mildly enlarged and contained moderate infiltrates of eosinophils and neutrophils. Blood, spleen, heart, skin and cervical and mesenteric lymph nodes tested positive for Ap by PCR, and sequence analysis confirmed infection of the lamb with the NY-18 isolate. Transplacental transmission should therefore be considered as a means of Ap transmission and may likely contribute to the epidemiology of tick-borne fever in sheep.

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Inactivation of bacteria in milk using a flow-through UV-light treatment system.

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The practice of feeding unpasteurized milk from sick cows receiving antibiotic treatment (“waste milk”) to dairy calves is common on dairy farms in the USA. The use of UV-light for the treatment of milk has the potential to reduce bacterial load in waste milk and decrease the dissemination of pathogens on the farm and throughout the food chain. This study evaluated the efficacy of a continuous tubular flow-through UV-light machine (GEA Farm Technologies, Inc) for the treatment of milk contaminated with bacteria. Autoclaved commercial whole milk was inoculated with Listeria innocua , Streptococcus agalactiae, Escherichia coli, Acinetobacter baumannii, Staphylococcus aureus and Salmonella typhimurium. Prior to each UV-light treatment, a milk sample was taken (PT) as a bacterial count reference. Once the UV-light treatment started milk samples were taken during the treatment and corresponded to the entire milk batch (4 liters) recirculating through the UV-light 40(T1), 80(T2) and 120(T3) times (65L/min flow rate). A minimum of four milk batch repetitions were done for each organism and bacteria were enumerated using serial dilution and plating on a selective solid agar media for each bacteria species (Chromagar tm, Dickinson Becton). The result for the Log10 mean reduction using the data obtained from all bacteria counts showed that from PT to T1 there was a 1.53- log10 CFU/ml reduction (95% CI:1.33- to 1.72- log10 CFU/ml reduction), from PT to T2 there was a 2.68 log10 CFU/ml reduction (95% CI: 2.46- to 2.89- log10 CFU/ml reduction) and from PT to T3 there was a 3.29-log10 CFU/ml reduction (95% CI: 3.01- to 3.57- log10 CFU/ml reduction). A multivariate analysis of variance (MANOVA) was used (JMP® Pro 9.0.2) to analyze the log10 CFU/ml reduction from PT to T1, PT to T2 and PT to T3 for each bacteria species studied. The results from this analysis showed a statistically significant log10 CFU/ml reduction from the effect of recirculating the milk through the UV-light for all six bacteria species (P-value<0.05). In conclusion we observed that the prototype machine using continuous tubular flow-through UV-light has the potential for inactivation of bacteria in autoclaved commercial whole milk.

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Temporal and spatial distribution of borreliosis, ehrlichiosis, anaplasmosis, and Rocky Mountain spotted fever in humans and dogs in Illinois from 2000-2009.

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Borreliosis, ehrlichiosis, anaplasmosis and Rocky Mountain spotted fever are tick borne bacterial diseases (TBD) capable of causing significant clinical signs in humans and dogs. Human cases of all four diseases have been reported in Illinois with increasing frequency over the past twelve years. However, there is no survey data on the incidence of these diseases among dogs in Illinois. The objective of the present study is to describe the temporal or spatial associations between human and canine cases and any effects of environmental factors on TBD incidence. To determine the number of human cases of TBD by residence and year, data from the Illinois Department of Public Health were used. To determine the number of canine cases, a survey was distributed to a random sample of veterinary clinics in Illinois. Additional canine case information was obtained through a follow-up survey of veterinarian respondents. The incidence of human and canine cases of all four vector borne diseases rose significantly over the study period with significant differences in geographic distribution. Most human cases were white, young to middle aged adult males and most were diagnosed during the second and third quarters of the year. Human Lyme cases showed a bimodal age distribution with most cases occurring in persons less than 20 and greater than 40 years of age. Most of the canine cases were middle-aged, hunting breeds and most were diagnosed from March through July. There was concordance in the number of human and canine cases by county of residence, in annual incidence trends and in time of year for diagnoses. Estimated annual incidence of canine TBD cases exceeded the number of reported human TBD cases by factors of 1.5 to 150 with the exception of Rocky Mountain spotted fever in the South region. Preliminary analysis of environmental data suggests an association between regional increases of temperature and precipitation and TBD incidence. Multivariate analysis of environmental data will be presented. The data suggest that dogs could be indicators of human disease and that veterinarians, physicians and public health agencies should communicate with each other regarding cases tick borne diseases.

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Evaluation of the systemic inflammatory reaction to anthelmintic treatment in ponies

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Grazing horses are widely exposed to infection with strongyle type parasites, infections which are largely controlled with administration of anthelmintic formulations to avoid parasitic disease. However, anthelmintic treatment can inadvertently induce inflammatory reactions and clinical disease. Very little research has been performed evaluating the inflammatory response to anthelmintic treatment, but one study indicates that treatment with moxidectin causes less of an inflammatory reaction than treatment with other drugs. Within the scope of this study, we aimed to explore the differences in inflammatory response following treatment with three different anthelmintic drugs: moxidectin, pyrantel pamoate, and oxibendazole. A population (n=30) of healthy, naturally parasitized ponies were allocated into the three treatment groups, based on age and worm fecal egg counts. All ponies were weighed and received the labeled anthelmintic dosage. Treatment efficacy was evaluated using the fecal egg count reduction test over a period of eight weeks, with weekly egg counts. The inflammatory response was assessed at four measuring points during the 14 days following treatment. Measurements involved characterization of cytokine gene expression and systemic inflammatory reaction. The objective of this study was to determine the effect of de-worming treatment on pro-inflammatory cytokine gene expression in the peripheral blood, and to evaluate any correlation between the expression of inflammatory cytokines with levels of acute phase proteins and inflammatory markers. Fecal egg counts from the study confirmed resistance levels in the parasite population. Treatment with oxibendazole and pyrantel pamoate was unsuccessful in the elimination of luminal parasites. Moxidectin, however, was very effective and egg counts of zero persisted for several weeks. Preliminary analysis of cytokine gene expression data shows a trend towards elevated levels of Interleukin-1beta in the moxidectin group; acute phase protein levels did not follow this trend, thereby negating the correlation between the two measurements of systemic inflammation.

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Recent advances in research of the Q fever bacterium, Coxiella burnetii

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Coxiella burnetii is gram-negative bacterium that causes the zoonotic disease Q fever. Symptomatic infections usually manifest as an acute, disabling influenza-like illness. The organism is highly infectious, environmentally stable, and usually transmitted to humans via inhalation of contaminated aerosols generated by animal husbandry operations. Dairy cows, sheep, and goats are important animal reservoirs of *C. burnetii*, with parturition by infected females depositing tremendous numbers of stable and highly infectious bacteria into the environment. *C. burnetti* is also a recognized biothreat. The past few years have witnessed significant gains in our understanding of *C. burnetii* genetics, virulence potential, and pathogen-host interactions. Comparative genomics reveal distinct strains of *C. burnetii* that display different pathogenicity for laboratory animals, suggesting a role for strain diversity in the natural history of human Q fever. Strains require full-length lipopolysaccharide for virulence; however, the molecule is non-stimulatory but instead appears to shield the organism from innate immune recognition. *C. burnetii* has uniquely evolved to replicate in the most inhospitable of cellular compartments, i. e., the phagolysosome of mononuclear phagocytes. Here, the organism undergoes luxurious growth that involves generation of developmental forms adapted to intracellular replication and extracellular survival. Understanding how *C. burnetii* resists the degradative functions of its replication vacuole, and the host cell functions co-opted for successful parasitism, are central to understanding Q fever pathogenesis. The organism deploys a type IV secretion system to deliver a complex collection of effector proteins directly into the host cell cytoplasm that modulate processes such as vesicular trafficking and apoptosis. Milestone discoveries of genetic transformation and host cell-free growth of this former obligate intracellular bacterium are currently enabling molecular dissection of type IV secretion and other virulence functions, and should aid development of a new generation of *C. burnetii* countermeasures.

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The ecology of eastern equine encephalitis virus in wildlife and mosquitoes in Minnesota

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Eastern equine encephalitis virus (EEEV) is a mosquito-borne zoonotic virus that is prevalent in North America. The primary transmission cycle involves mosquitoes and wild birds as reservoir hosts and infection has been documented in horses,humans and wildlife species. The aim of this correlative study was to examine vectors of EEEV in relation to high antibody titers found in moose and elk that were sampled as part of the Minnesota Department of Natural Resources' wildlife health surveillance efforts.

Adult mosquitoes were sampled weekly during the summer of 2012 from 6 regions in northern Minnesota. In each region, one trapping site was in an area with high moose or elk seroprevalence, while another was in a matching low seroprevalence area. Specimens were identified to species by light microscopy and taxonomic key.

Preliminary results indicate that both Aedes sticticus and Aedes vexans were more abundant in high seroprevalence trapping locations. Culiseta melanura, the enzootic amplifying vector has not been obtained in any of our 6 trapping regions.

Utilizing these findings along with previous studies, we can prioritize which species to test for EEEV by RT-PCR. After testing, if infection prevalence is sufficient, regression methods will be employed to determine which factor, or combination of factors: mosquito species, region, or time is the most predictive of wildlife exposure status. When this project is complete a more effective approach to targeting mosquito populations that transmit the virus can be developed to protect the spread of disease to susceptible populations.

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Anthelmintic effect of proanthocyanidin extract of cranberry leaf powder on *Haemonchus contortus* and *Caenorhabiditis elegans*

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Purpose: Emergence of anthelmintic resistance in all species of gastrointestinal nematodes, particularly *Haemonchus contortus* (*H. contortus*), and growing concern over chemical residues in animal products and in the environment has made the development of alternative methods of parasite control for small ruminants vital. One of the most promising findings, in the last fifteen years in the search for alternative methods of gastrointestinal nematode (GIN) control, has been the discovery that consumption of some forages containing condensed tannins, also called proanthocyanidins (PAC), suppress GIN infection. The objective of this study was to investigate the anthelmintic potential of the PAC contained in cranberry leaves. Methods: The effect of PAC on the viability of adult *Caenorhabiditis elegans* (*C. elegans*) and larval *H. contortus* were tested using *in vitro* methods: 1) Adult *C. elegans* worms grown in axenic medium were collected with sieves and exposed to varying concentrations of PAC extract. Nematodes were examined after 24 hr incubation and classified as dead or alive based on motility, 2) After hatching, L1/L2 *H. contortus* larvae were incubated in 1 mg/mL PAC or water for 24 hr and classified as dead or alive based on motility and, 3) The effect of PAC on exsheathment of L3 *H. contortus* larvae was also tested. Larvae were exposed to 0.5 or 1.0 mg/mL PAC for 3 hr, after which larvae were washed with water, exposed briefly to carbon dioxide and incubated for 18 hr at 37 C. Larvae were counted and classified as exsheathed or not exsheathed. Results: 1) At 1, 5, 10, 20 and 25 mg/ml the number of live adult *C. elegans* was reduced by 70%, 93%, 98%, 100% and 100%, respectively, 2) At 1 mg/mL PAC the number of live L1/L2 *H. contortus* larvae was reduced by 67%, 3) There was no effect of 3 hr incubation of PAC on L3 exsheathment. Conclusions: Cranberry leaf powder PAC exhibited anthelmintic activity against adult *C. elegans* and *H. contortus* L1/L2 larvae after 24 hr incubation but there was no effect on exsheathment of *H. contortus* L3 after a 3 hr incubation. Further *in vitro* and *in vivo* studies are warranted to determine the efficacy of cranberry PAC for the control of GIN in small ruminants.

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The chlamydiosis pathogenesis studies at experimental infection of white rats

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Grey rats being synanthropic animals are considered to be the vector of Chlamydia transfer to domestic animals. Objective of investigation was to study Chlamydiosis pathogenesis in experimental infection in white rats. 88 white rats were intranasally and intraperitoneally inoculated with field isolates: C. abortus - isolated from pigs, C. psittaci - from birds, C. psittaci - from grey rats and C. pecorum - from domestic and wild carnivora. The mentioned isolates displayed high virulence at bioassays on white mice and guinea pigs. The control group included 22 rats. The clinical state of all rats was within the physiological norms, with the exception of one rat died on 16 day after infection with symptoms of general depression, exhaustion and apastia. In most of the infected animals we registered the rise of body temperature 0.2-0.3°C on 3-7 d.a.i., but the physiological norm 38°C was not exceeded. Pathology studies of the most animals, euthanized on 14 and 21 d.a.i., demonstrated the venous stasis of cerebral vessels, areas of catarrhal and catarrhal-haemorrhagic pneumonia in lungs and hypertrophy of spleen and liver. In animals euthanized later, no visible pathologic changes were registered. The microscopy revealed elementary bodies on the Stamp stained smears from cerebral, lungs, spleen and liver. The intensity of the Chlamydial infection reached its maximum on 14 d.a.i. Microscopy of smears taken from animals euthanized later showed the negative dynamics of the infection intensity till the absolute absence of inclusion bodies. PCR of the 20% organ samples revealed the chlamydial DNA in all samples from infected rats, euthanized in 10-30 d.a.i., and in separate animals, culled on 35-49 d.a.i. Both microscopy and PCR revealed no positive results in organ samples from rats killed on 55 d.a.i. The results demonstrate that the infection process intensity in the rat's organism reaches its maximum on 14-21 d.a.i. and then gradually goes down to the absolute elimination of Chlamydia on 42-49 d.a.i. These results lead to the conclusion that murine rodents of the rat genus are nondurable vectors and, consequently, they cannot play any significant role in Chlamydial agent's transfer to domestic animals.

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A novel small structural protein ORF5a is essential for porcine reproductive and respiratory syndrome virus production

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A novel ORF5a protein, has been recently described as being expressed by an alternative open reading frame of subgenomic RNA 5 in all arteriviruses including porcine reproductive and respiratory syndrome virus (PRRSV). In the case of equine arteritis virus (EAV), an ORF5a knock-out mutant was successfully generated, although its replication and virus yield were seriously impaired. This suggested that the ORF5a protein may be dispensable for arterivirus replication. We attempted a series of knock-out mutations of PRRSV ORF5a using an infectious clone FL12. We ensured that ORF4 and 5 coding regions remained untouched in these constructs by making the mutations within the intergenic junction (10 nucleotides long between ORF4 and 5 in case of North American strains). By immunofluorescence, we could observe frank evidence of single round replication in either MARC-145 or BHK-21 cells at 48 hrs post electroporation and/or combined with passing into peripheral blood mononuclear cell (PBMC) derived macrophages. Nonetheless, we failed to recover any viable mutant virus containing a correctly knocked out ORF5a gene. In some instances, however, we were able to recover viable viruses long after electroporation and/or passage but all these rescued viruses turned out to be wild type by sequence analyses. This suggests that there must be a strong selective pressure in ORF5a encoding sequences, especially within the intergenic junction, and that ORF5a may not be dispensable for PRRSV replication.

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Virion packaging of multiple cleavage isoforms of porcine reproductive and respiratory syndrome virus nonstructural protein 2

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Porcine reproductive and respiratory syndrome virus (PRRSV) is the cause of a complex disease often resulting in significant morbidity and mortality. Recently, highly pathogenic isolates have emerged which have proven to be devastatingly effective pathogens, resulting in rapid systemic deterioration of the host. PRRSV exhibits a profound ability to evolve in response to immunological pressures by both mutation and recombination events. Due to the substantial genetic heterogeneity between strains, identifying the underlying molecular mechanisms utilized by PRRSV to support infection has been challenging. The replicase nonstructural protein 2 (nsp2) is both the largest and the most genetically diverse viral protein. Nsp2 is a multidomain protein, with a recognized Ovarian Tumor (OTU)-domain protease (PLP2) near its N-terminus, a long hypervariable region, a transmembrane region and a relatively conserved C-terminal domain. The function(s) of nsp2 have not been well defined but are believed to include proteolytically cleaving the nsp2-nsp3 junction, a suspected deubiquitinating activity, a role as a scaffolding protein supporting the replication machinery, as well as antagonistic immunomodulatory properties targeting the innate immune system. Our investigation of highly purified PRRSV revealed the identification of nsp2 within the virion of multiple diverse strains by both immunoelectron microscopy (IEM) and western blot analysis. Western blot analysis identified cleavage isoforms between approximately 120kDa to 50kDa packaged into the virion of multiple strains. IEM and western blot results were consistent across genetically diverse strains including European Type 1 and the North American Type 2 prototype strains Lelystad and VR-2332 respectively, as well as highly pathogenic Asian strains JXwn06 and SRV-07. The strong antigenicity of nsp2 has been shown to result in the generation of significant α-nsp2 antibody titers *in vivo*. The identification of nsp2 incorporation into the virion may partially explain the selective pressure underlying the robust plasticity of nsp2. This represents the first report of the incorporation of nsp2 within the PRRSV virion.

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Host cell gene expressions and cell cycle progression regulated by PRRS virus Nsp11 protein

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PRRS virus Nsp11 is a vial endoribonuclease that is essential for infectivity with an unknown mechanism. To examine the cellular gene expression profiles regulated by Nsp11, MARC-Nsp11 cells were constructed using retrovirus-mediated gene transfer for the stable expression of Nsp11, and an RNA microarray was conducted in these cells. In the MARC-Nsp11 cells, the IFN-β, IRF3, and NF-κB promoter activities were suppressed compared to those of the MARC-145 cells, indicating that Nsp11 is an IFN antagonist of PRRSV and retains its regulatory role in MARC-Nsp11 cells. Differential host cell transcription profiles regulated by Nsp11 were then examined using Affymatrix exon chips representing 28,536 human gene transcripts. After statistical analyses, 66 cellular genes were shown to be up-regulated and 104 genes were down-regulated. These genes were further examined and grouped into 5 major cellular pathways according to their functional relations: histone-related, cell cycle and DNA replication, mitogen activated protein kinase signaling, complement, and ubiquitin-proteasome pathways. Of these, the modulation of the cell cycle was further examined. Flow cytometry analysis showed that Nsp11 caused the inhibition of cell cycle progression, and the BrdU staining for DNA in replicating cells indicated slower progression through the S-phase. Our study shows that the PRRSV Nsp11 protein contains an ability to modulate the host cell cycle progression and provides insights into specific cellular responses to Nsp11 during infection.

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Suppression of host gene expression by nsp1β protein of porcine reproductive and respiratory syndrome virus

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Previous studies from our laboratory and others identified the nonstructural protein 1β (nsp1β) of porcine reproductive and respiratory syndrome virus (PRRSV) as a strong interferon antagonist. In this study, nsp1β was found to suppress host gene expression by promoting host mRNA degradation and inhibiting translation. Expression of nsp1β prevented Sendai virus-induced endogenous IFN-β mRNA accumulation and also promoted the degradation of expressed RNA transcripts and endogenous GAPDH mRNA, resulting to a strong inhibition in host protein synthesis. In contrast, expression of nsp1β did not affect the accumulation of 28S and 18S rRNAs. In an effort to remove the effect of nsp1β on host gene expression, a panel of site-specific nsp1β mutations was analyzed. The K130A/R134A double mutation, targeting on a highly conserved motif on the protein, impaired the ability of nsp1β to suppress host gene expression. The nsp1β-K130A/R134A was neither promoted host mRNA degradation nor suppressed host protein synthesis in nsp1β- expressing cells. The data indicate that PRRSV nsp1β promotes host mRNA degradation and thereby suppresses host gene expression, including proteins involved in host innate immune functions. This suggests that nsp1β could be a virulent factor and play an important role in PRRSV pathogenesis.

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The PRRSV-mediated inhibition of interferon alpha production by its natural host cell occurs at the post-transcriptional level.

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Research on the ability of porcine reproductive and respiratory virus (PRRSV) to inhibit the host interferon (IFN)-α response has focused mainly on the effect of individual viral proteins on transcription factors involved in the type I IFN response. By using non-PRRSV natural host cells transfected with reporter gene constructs in combination with over-expressed viral proteins, this type of studies have indicated that several nonstructural proteins of PRRSV have the ability to negatively affect the activation of NFκB and IRF-3. Concurrently, there is convincing evidence indicating that live PRRSV is indeed able to hinder the ability of its natural host cell, namely porcine alveolar macrophages (PAMs), to produce IFN-α in response to their stimulation with strong agonists, such as the synthetic analog of dsRNA, poly(I:C). Efforts to ascertain the mechanism(s) by which the infection of PAMs with PRRSV hinders the type I IFN response have been stifled by the fact that only a small fraction of this cell population is susceptible to infection by this virus. Consequently, any effect that PRRSV might have on transcription factor activation resulting from stimulation with poly(I:C) is obscured by the majority of the cells that are responding to this agonist, but are not infected

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by PRRSV. Here, we evaluated the effect of PRRSV on the poly(I:C) stimulated activation of the transcription factors IRF-3 and NFκB, IFN-α and IFN-β gene transcription as well as IFN-α secretion by ZMAC cells. The ZMAC cell line is a non-transformed PAM that, as a population, is readily and 100% susceptible PRRSV infection and has an intact type I IFN response system. Our results demonstrate that infection of ZMAC cells with PRRSV does not inhibit the poly(I:C)-induced activation of NFκB, STAT-1 or IRF-3, nor does it inhibit IFN-α or IFN-ß gene transcription. Nevertheless, the secretion of IFN-α was inhibited by >60% by 9 hours after infection. Notably, PRRSV alone induced the phosphorylation of NFκB but not IRF-3. Accordingly, PRRSV did not induce secretion of IFN-α. Our results indicate that whatever the mechanism is by which PRRSV inhibits the secretion of IFN-α in PAMs, it occurs at the post-transcriptional level.

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Variable interference with interferon signal transduction by different PRRSV strains
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Porcine reproductive and respiratory syndrome virus (PRRSV) interferes with interferon (IFN)-activated antiviral response. PRRSV nsp1β appears responsible for the interference with IFN-activated signaling. In this study, different PRRSV strains of various virulence were compared to reveal their effects on IFN signal transduction pathway. One strain of genotype 1 PRRSV (LeLystad) and five strains of genotype 2 (VR-2385, MLV, VR-2332, NVSL, and MN-184) were used to infect MARC-145 cells. Compared to uninfected cells, all of the strains except MN184 led to much lower IFN-induced STAT2 protein expression and reduced the transcript level of IFN-stimulated genes (ISGs), ISG15 and ISG56, in infected MARC-145 cells. In primary porcine alveolar macrophages (PAMs), all strains except MLV and NVSL inhibited IFN-induced elevation of STAT2 protein. PRRSV A2MC2, an IFN-inducing strain, enhanced STAT2 expression in both MARC-145 and PAM cells and was included as a control. Nsp1β sequences from these strains were cloned into an expression vector and used to compare their effect on IFN signaling. Analysis of ISG15 mRNA level in HEK293T cells transfected with the different nsp1β plasmids showed variable interference with IFN-induced ISG expression. Further work is undergone to delineate the different effect of these strains on IFN-activated signal transduction.

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Identification of regulatory domain of PRRS virus nonstructural protein 1 alpha for type I interferon modulation
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The PRRS virus non-structural protein (Nsp) 1 has been shown to be a type I IFN antagonist of porcine reproductive and respiratory syndrome virus (PRRSV).The CREB-binding protein (CBP) is degraded in the presence of Nsp1, which is likely the basis of the IFN suppression. Nsp1 is autocleaved to Nsp1α and Nsp1β subunits, and in the present study, we found that the Nsp1α subunit was responsible for CBP degradation. To study the structure function relationship of the Nsp1α subunit, papain-like cysteine protease (PCP) α, zinc finger (ZF) 1, and ZF2 motifs were examined by mutational analyses for their ability for IFN suppression in the luciferase reporter assay. Nsp1α-C76S, Nsp1α-H146Y, and Nsp1α-C76S/H146Y maintained the IFN suppressive activity, indicating the protease activity of PCPα does not participate in the IFN suppression. Single mutations and double mutations at residues of C70, C76, H146, and M180 coordinating ZF2 did not change the IFN suppressive activity, showing that ZF2 was also not involved in the IFN down-regulation. Single and double mutations for residues at C8, C10, C25 and C28 of ZF1 were found to impair the IFN suppressive activity indicating that ZF1 was the element important for IFN suppression. The ZF1 mutant proteins did not localize to the nucleus by immunofluorescence, and the CBP protein was not degraded by the ZF1mutants. Taken together, our data show that the ZF1 motif of Nsp1α plays a key role for IFN regulation during PRRSV infection.

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PRRSV nsp1β inhibits interferon signal transduction by inducing importin-α5 degradation
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Porcine reproductive and respiratory syndrome virus (PRRSV) interferes with interferon (IFN) signal transduction pathway to antagonize innate antiviral response. Type I IFNs induce the expression of IFN-stimulated genes by activating phosphorylation of both the signal transducer and activator of transcription 1 (STAT1) and STAT2, which form heterodimers, interact with IRF9 and translocate to the nucleus. PRRSV nsp1β blocks the nuclear translocation of the heterotrimer by an unknown mechanism. The objective of this study was to explore the mechanism of nsp1β in inhibition of the heterotrimer nuclear translocation. Here we discovered that nsp1β induces degradation of karyoperin-α1 (KPNA1, also known as importin-α5), which is essential for the nuclear translocation of STAT1. Overexpression of nsp1β led to reduction of KPNA1 protein level but had no effect on its transcript level. Addition of a proteosome inhibitor restored the KPNA1 protein level. Presence of nsp1β shortened half-life of KPNA1. Immunoprecipitation did not reveal interaction between nsp1β and KPNA1. Analysis of nsp1β deletion constructs showed that the amino half of nsp1β involved in the degradation of KPNA1. Interestingly, nsp1β of Ingelvac PRRS MLV had no effect on KPNA1. A point mutation of one nucleotide near 5'end of nsp1β of VR-2385 abolished its ability to induce degradation of KPNA1 and inhibit the expression of interferon-stimulated genes. Infection of MARC-145 cells by PRRSV VR-2332 and VR-2385 led to reduction of KPNA1, while MLV had no effect. These results indicate that nsp1β interferes with IFN signal transduction via inducing degradation of KPNA1. This discovery provides further insight of PRRSV interference with innate immunity.

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The disease manifestations of two Asian highly pathogenic strains of Type 2 PRRSV
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Highly pathogenic Type 2 PRRSV isolates (HP-PRRSV) have been circulating in Asia for 6 years. rJXwn06 and rSRV07 were rescued from infectious clones of two Asian HP-PRRSV isolates for use at the National Animal Disease Center. The clinical disease and viral replication

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kinetics of the viruses were compared to the North American prototype strain VR-2332. Four-week-old pigs were inoculated intranasally with either a low (2 x 10^3 TCID50) or high (2 x 10^6 TCID50) dose of the rJXwn06, rSRV07, or VR-2332 isolate. For 13 days post-inoculation (dpi), swine were monitored for clinical disease and samples collected to assay virus load and host cytokine response. Control swine were inoculated with a virus-free cell culture medium. Following inoculation with rJXwn06, a rapid onset of disease (fever, weight loss, respiratory distress) occurred that led to death or euthanasia in all low and high dosed pigs by 11 dpi. The disease in the low and high dosed rSVR07 inoculated swine was similar, but less intense over the duration of the study. The onset was delayed 1-2 days and there was less weight loss, respiratory distress, and mortality. In general, the high dose pigs were more affected than the low dose pigs. In comparison, the VR-2332 swine were mildly affected. The HP-PRRSV inoculated pigs had significant changes in their innate and adaptive cytokine responses when compared to the VR-2332 and control pigs, indicating a strong immunomodulatory effect of the Asian HP-PRRSV isolates. This is supported by the isolation of a number of bacterial species from the HP-PRRSV affected pigs in contrast to little or none isolated from the VR-2332 and control swine. In another study, the use of an attenuated PRRSV vaccine (Ingelvac PRRS® MLV) to protect pigs from HP-PRRSV infection was evaluated. Pigs were vaccinated at 4-weeks-of age and at 10-weeks-of age received an intranasal challenge with a high dose of either HP-PRRSV isolate. Vaccination reduced the clinical effect of the HP-PRRSV challenge when compared to non-vaccinated challenge controls. However, many of the vaccinated swine became very sick with some mortality in the rJWxn06 challenge groups. No disease was recognized in the vaccinated/VR-2332 inoculated swine.

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Comparison of Asian highly-pathogenic PRRSV isolates to US isolates for their ability to cause secondary bacterial infection in swine
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The appearance of highly-pathogenic porcine reproductive and respiratory syndrome virus (PRRSV) isolates in Asia necessitates investigation into the clinical repercussions of these viruses if the strains were to appear in the US. Epidemiologic data from Asian outbreaks suggest that disease severity was associated with both the PRRSV isolates from these cases and secondary bacterial infections. Previous reports have indicated that US isolates of PRRSV predispose to secondary bacterial infections as well, but outbreaks like the ones described in Asia have not been reported in the US. The objectives of this research were to compare the pathogenesis of Asian and US PRRSV isolates of varying virulence with regard to their ability to cause disease and predispose to secondary bacterial infections in swine. The experiment consisted of 10 groups of 9-10 pigs each. At 6 weeks of age, half the groups were inoculated with a bacterial cocktail of *Streptococcus suis*, *Haemophilus parasuis*, and *Actinobacillus suis*and 1 week later 4 bacterial colonized groups and 4 non-bacterial colonized groups were inoculated with 1 of 2 Asian HP-PRRSV strains (JXwn06 or SRV07) or 1of 2 US PRRSV strains (SDSU73 or VR2332). The pigs infected with JXwn06 were clinically the most severely affected (based on clinical signs, febrile response, and weight gain) while the pigs infected with SRV07 and SDSU73 were moderately affected, and pigs infected with VR2332 showed minimal clinical signs. One pig coinfected with JXwn06 and bacteria had to be euthanized. The highest viral titers were detected in pigs challenged with JXwn06. *A. suis* and/or *H. parasuis* was cultured from the lungs of 3/9 pigs from groups challenged with the bacteria alone, VR2332/bacteria, and SDSU73/bacteria, and from 6/9 pigs challenged with SVR07/bacteria and JXwn06/bacteria, respectively. These bacteria were not isolated from the non-challenged control pigs or pigs challenged with virus alone. Lesions consistent with bacterial pneumonia, including abscesses, were seen in the groups coinfected with PRRSV and bacteria. There was a range of virulence among the PRRSV isolates and differences in their ability to predispose to secondary bacterial infection

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Changes in circulating and thymic lymphocyte populations following infection with strains of North American or Highly Pathogenic PRRSV.
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Recently, a highly pathogenic (HP) PRRSV strain has emerged in Asia, which causes severe clinical disease and mortality. Since its emergence, work has focused on characterizing the virus and host response following infection to determine the mechanism of enhanced virulence. PRRSV infection has been shown to cause a decrease in circulating T cell populations, lymphadenopathy and thymic atrophy; however, the relationship between these features in relation to HP-PRRSV has not been evaluated. Groups of pigs were challenged with one of two different North American isolates (VR-2332 or SDSU73) or one of two different HP-PRRSV isolates (SRV07 or JXwn06) for this study. Circulating T cell populations were enumerated on 1-4, 6, 8 and 10 days post-infection (dpi) using a newly developed flow cytometric based assay with whole blood. T-cell populations in the thymus and lymph node were evaluated on dpi 4 and 10. Regardless of the challenge strain, there was a significant decrease in the number of circulating CD3+ T-cells, including CD4 and CD8 subsets, following infection. The sharpest decline occurred between dpi 1 to 2 in VR-2332, SDSU73 and JXwn06 groups and between dpi 2 to 4 for SRV07 group. There was not a significant difference in the lowest number of circulating T cells between the SDSU73, SRV07 or JXwn06 groups. VR2332, SDSU73 and JXwn06 groups were viremic by dpi 1 while pigs challenged with SRV07 displayed a gradual increase in serum virus titers. The JXwn06 group had the greatest amount of virus in the sera beyond dpi 2; however, the number of circulating T-cells was similar between SDSU73 and JXwn06 groups. Thus, serum virus titers alone do not explain the decrease in circulating T-cells. There was a significant increase in the number of dead T-cells (CD4, CD8 and CD4/CD8) in the thymus of all PRRSV infected pigs, though SDSU73 and JXwn06 pigs were the most affected. There was not a significant increase in serum cortisol levels in any of the pigs. Taken together, there is a rapid decrease in the number of circulating T-cells following PRRSV infection, but, this effect does not appear to correlate to virulence, serum virus titers nor serum cortisol levels.

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Swine tracheobronchial lymph node mRNA responses in swine infected with a highly pathogenic strain of Porcine Reproductive and Respiratory Syndrome virus.
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Purpose: Porcine reproductive and respiratory syndrome virus (PRRSV) is a major pathogen of swine worldwide. Emergence in 2006 of a novel highly pathogenic PRRSV (HP-PRRSV) isolate in China warranted a comparative investigation into the host transcriptome response in tracheobronchial lymph nodes (TBLN) 14 days post-infection with HP-PRRSV rJXwn06, strain VR-2332 or sham inocula.
Methods: RNA from each was prepared for next-generation sequencing. Amplified library constructs were directly sequenced and a list of sequence transcripts and counts was generated using an RNAseq analysis pipeline to determine differential gene expression. Transcripts were annotated and relative abundance was calculated based upon the number of times a given transcript was represented in the library.
Results: The largest increase in transcript level for either virus versus sham-inoculated controls were three serum amyloid A2 acute-phase isoforms. However, the degree of up or down-regulation of transcripts following infection with HP-PRRSV rJXwn06 was greater than transcript changes observed with US PRRSV VR-2332. Also, of 632 significantly altered transcripts within the HP-PRRSV rJXwn06 library 55 were upregulated and 69 were downregulated more than 3 fold, whilst in the US PRRSV VR2332 library only 4 transcripts were upregulated and 116 were downregulated more than 3 fold.
Conclusions: The magnitude of differentially expressed gene profiles detected in HP-PRRSV rJXwn06 infected pigs as compared to VR-2332 infected pigs was consistent with the increased pathogenicity of the HP-PRRSV in vivo.

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Attenuation of porcine reproductive and respiratory syndrome virus by molecular breeding of the virus envelope genes from genetically divergent strains

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Molecular breeding via DNA shuffling can direct the evolution of viruses with desired traits. By using a positive-strand RNA virus, porcine reproductive and respiratory syndrome virus (PRRSV), as a model, rapid attenuation of the virus is achieved in this study by DNA shuffling of the viral envelope genes from multiple strains. The GP5 envelope genes of 7 genetically divergent PRRSV strains and the GP5-M genes of 6 different PRRSV strains were molecularly bred by DNA shuffling and iteration of the process, and the shuffled genes were cloned into the backbone of a DNA-launched PRRSV infectious clone. Two representative chimeric viruses, DS722 with shuffled GP5 genes and DS5M3 with shuffled GP5-M genes, were rescued and shown to replicate at a lower level and formed smaller plaques in vitro when compared to its parental virus. An in vivo pathogenicity study revealed that pigs infected with the two chimeric viruses have significant reductions in viral RNA loads in sera and lungs, and in gross and microscopic lung lesions, indicating attenuation of the chimeric viruses. Furthermore, pigs vaccinated with the chimeric virus DS722, but not with DS5M3, still induced protection against PRRSV challenge at a level similar to that of its parental virus. Therefore, this study reveals a unique approach through DNA shuffling of viral envelope genes to rapidly attenuate a positive-strand RNA virus. The results have important implications for future vaccine development and will generate broad general interest in the scientific community for rapidly attenuating other important human and veterinary viruses.

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Development of a modified live vaccine against porcine reproductive and respiratory syndrome with optimal "DIVA" marker potential

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While still subject to improvement, live vaccines are well accepted as the most effective tool for control and eradication of porcine reproductive and respiratory syndrome (PRRS). One major limitation of current PRRS vaccines is that they do not allow serological discrimination between naturally infected andvaccinated pigs (DIVA). Since the initial application of DIVA vaccines in pigs to eradicate Pseudorabies Virus (Suid Herpesvirus 1), epidemiological as well as regulatory considerations dictate that a DIVA vaccine should be designed based on a "negative" marker. A marker is a viral protein or an epitope absent (thus "negative") from the vaccine strain but consistently present in wild-type strains. Therefore, only animals that have been infected with wild type virus should develop antibodies against the marker epitope while the vaccinated animals should not. Antibody reaction against the marker epitope is the indicator of infection with wild-type virus. Here, we report the development of a modified live vaccine against PRRS with optimal DIVA marker potential. We had previously identified several immunodominant B-cell linear epitopes in the proteins of a type-II PRRSV strain FL12, of which, the epitope number 201 (EP-201) located at the carboxyl terminal region of the M protein was selected as a marker candidate. Comparison of the amino acid sequence of ~100 M proteins collected from the NCBI database revealed that this epitope is highly conserved across the type-II PRRSV strains. Moreover, a monoclonal antibody specific to EP-201 (anti-201 MAb) recognized 92% (n=81) type-II PRRSV isolates, confirming the conservation of this epitope. A mutant virus FL12-TM carrying 3 amino acid substitutions in the EP-201 region of PRRSV FL12 was generated by site-directed mutagenesis. The FL12-TM was no longer recognized by anti-201 MAb in the indirect immune-fluorescent assay. More importantly, pigs infected with FL12-TM developed significant lower levels of antibody response to EP-201 as compared to those infected with wt FL12, indicating the potential use of FL12-TM a DIVA marker vaccine strain. Current emphasis is directed at optimization of the EP-201 ELISA companion assay.

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Flexible polymer adjuvants for live and inactivated vaccines: Application to PRRS live vaccine

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Purpose: Live vaccines are widely used in pig farming practice and are usually not adjuvanted. In this study we show that the addition of the polymeric adjuvant Montanide™ Gel 01 in a PRRS live vaccine enhanced the protection to challenge of vaccinated animals, and allowed to reduce the antigenic load of such vaccine while preserving its efficacy. As this adjuvant has been shown to be an efficient adjuvant for inactivated vaccines, it could also allow the formulation of combined live/inactivated vaccines. Methods: Live PRRS vaccines (North American genotype) were formulated extemporary in dilutant with no adjuvant, with the polymeric adjuvant Montanide™ Gel 01 at 10%. Each vaccine contained

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either 50% or 100% of the commercial dose (4.3 log TCD50/ml virus titer). A non vaccinated group was used as negative control. At day 0, 10 PRRS negative pigs (15 kg) were vaccinated in each group intramuscularly in the neck with 2ml of vaccine. Efficacy was followed by antigen specific ELISA and by a challenge procedure (day 30). After challenge clinical signs were followed and bacterial over-infections of the lungs were scored. Results: All vaccines tested were safe. Protection to challenge was significantly superior for adjuvanted formulations containing 100% of antigen compared to the non adjuvanted vaccine. For both types of adjuvants, despite lower antibody titers, the protection to challenge given by the adjuvanted vaccine containing only 50% of the antigen load was equivalent to the protection given by the non-adjuvanted vaccine. Conclusions: These results demonstrate that relevant aqueous adjuvants such as Montanide™ Gel 01 can enhance the efficacy of the protection conferred to animals by live vaccines and allow to reduce the antigenic dose of the vaccine. Such adjuvanted live vaccines could be combined with inactivated formulations.

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Novel simian hemorrhagic fever viruses from wild African primates offer new insights into the evolutionary origins of PRRSV
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Purpose: PRRSV is considered to be one of the most quickly evolving viral pathogens, having entered the domestic pig populations of Europe and North America nearly simultaneously and evolving rapidly to cause similar clinical disease on both continents. Unfortunately, our knowledge of PRRSV evolution is limited by lack of comparative information on the natural history and evolution of the other arteriviruses. Here we describe the discovery and characterization of novel simian hemorrhagic fever virus (SHFV) variants from wild African primates.
Methods: Blood plasma from 66 wild African primates was subjected to metagenomic analysis of total RNA for virus discovery. Complete genomes of four novel SHFV variants were recovered after de novo assembly of raw sequence reads and subsequent gap-filling.
Results: The novel SHFV variants are monophyletic and share characteristic genomic architectural features, including the presence of at least three unique open reading frames (ORFs) immediately downstream of the replicase-encoding ORFs. Prevalence data suggest that these viruses can establish persistent, high-titer infections. Metagenomic analyses suggest that SHFV infection may be facilitated by co-infection with multiple SHFV variants and other diverse RNA viruses.
Conclusions: Phylogenetic diversity among SHFV variants is greater than that observed for any other arterivirus, with a topology suggesting host restriction and ancient viral diversification. These results demonstrate that arteriviruses in nature can exist as metapopulations of highly divergent variants subclinically and persistently infecting hosts. This finding, in turn, raises the possibility that the evolutionary origins of PRRSV may be more ancient than commonly thought. Taxonomic reclassification of the arteriviruses is warranted.

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Validation of an equine arteritis virus antibody cELISA according to OIE protocol.
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Equine arteritis virus (EAV) is the cause of Equine Viral Arteritis characterized by conjunctivitis, nasal discharge, dependent edema, abortion, and infrequently, death in young foals. In attempting to produce a better alternative assay, a cELISA was developed using EAV gp5-specific non-neutralizing monoclonal antibody (MAb) 17B7, and validated according to the OIE-recommended validation protocol. As part of an in-house validation procedure of the EAV antibody cELISA, the following five analyses were performed: 1. the primary assay was calibrated with the OIE approved reference serum panel for EVA, 2. repeatability of the assay was evaluated within and between runs, 3. analytical specificity was evaluated using sera specific to related viruses, 4. analytical sensitivity was evaluated with sera collected from horses vaccinated with the modified live virus vaccine against EVA (Arvac®, Pfizer Animal Health), and 5. the duration of the positive cELISA antibody detection was evaluated following EVA vaccination. The outside validation of the cELISA utilized three laboratories including one OIE reference laboratory and two AAVLD-accredited state laboratories. Each laboratory assayed their panel of field sera (150-200 sera) to evaluate diagnostic specificity and sensitivity of the cELISA, and VMRD prepared an inter-dependency panel (25 sera in duplicate) to evaluate the robustness of the cELISA in all three laboratories. The cut-off of the cELISA was evaluated by ROC plot analysis. As a result, the analytical sensitivity of the new cELISA was comparable to the SN assay in that it detected EAV-specific antibody as early as 6 days post-vaccination. The duration of EAV-specific antibody detected by cELISA was over six years post-vaccination. In the field trial, the relative specificity of the new cELISA was 99.5% and the relative sensitivity was 98.2%. This field trial data also showed a significant correlation between SN and cELISA results (r^2=0.79, P<0.0001). These results indicate that new EAV antibody cELISA is a reliable, simple alternative to the SN assay for detecting EAV-specific antibodies in horses.

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Isolation of a novel swine influenza virus distantly related to influenza C
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Of the *Orthomyxoviridae* family of viruses, only influenza A is thought to exist as multiple subtypes and has non-human maintenance hosts. In April 2011, nasal swabs were collected for virus isolation from pigs exhibiting influenza-like illness. Subsequent electron microscopic, biochemical, and genetic studies identified an orthomyxovirus with seven RNA segments exhibiting approximately 50% overall amino acid identity to human influenza C virus. Based on its genetic organizational similarities to influenza C viruses this virus has been provisionally designated C/Oklahoma/1334/2011 (C/OK). Phylogenetic analysis of the predicted viral proteins found that the divergence between C/OK and human influenza C viruses was similar to that observed between influenza A and B viruses. No cross reactivity was observed between C/OK and human influenza C viruses using hemagglutination inhibition (HI) assays. Additionally, screening of pig and human serum samples found that

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9.5% and 1.3%, respectively, of individuals had measurable HI antibody titers to C/OK virus. C/OK virus was able to infect both ferrets and pigs and transmit to naive animals by direct contact. Cell culture studies showed that C/OK virus displayed a broader cellular tropism than a human influenza C virus. These results show that C/OK virus represents a new subtype of influenza C virus that currently circulates in pigs that has not been recognized previously. The presence of multiple subtypes of co-circulating influenza C viruses raises the possibility of reassortment and antigenic shift as mechanisms of influenza C virus evolution.

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Harnessing RNAi to inhibit avian influenza replication in avian cells using a novel delivery technology: Progressing towards an alternative prevention strategy.
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Outbreaks of avian influenza virus (AIV) have severe economic consequences to the poultry industry and increase the risk for transmission to humans. Current vaccination strategies are limited and clinical application of RNAi needs to be demonstrated. Transkingdom RNAi (tkRNAi), a unique delivery platform, uses nonpathogenic bacteria to generate and deliver siRNAs to target tissues. These novel tkRNAi vectors could be the key to attaining clinical application and our long term goal of using RNAi to develop a novel alternative to the AIV vaccine for commercial use in poultry. The objective was to provide proof of concept for inhibiting AIV using the novel tkRNAi platform to develop an anti-AIV vector to deliver siRNAs to chicken epithelial cells, as a preliminary avian tissue model for future work in chickens. The anti-AIV vectors were constructed and two specific aims were pursued: 1) Test vector uptake and invasion of chicken epithelial cells using a fluorescent marker; and 2) evaluate the anti-AIV vectors for their ability to inhibit AIV replication in chicken epithelial cells. Assessment of vector uptake and invasion, AIV replication, and the production of infectious viral particles were determined via flow cytometry, RT-qPCR based on the AI matrix gene, and TCID50, respectively. The anti-AIV vectors were efficiently delivered to chicken epithelial cells and these vectors show potential to inhibit AIV replication in vitro. Demonstrating the value of this novel approach could translate into an effective antiviral technology that limits outbreaks in poultry, and could represent a transformative approach to controlling influenza with great potential to have a sustained and significant impact on human disease. Applying the tkRNAi delivery approach is innovative and is the first instance of such a delivery mechanism to inhibit influenza replication.

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Pathogenicity and transmissibility of novel reassortant H3N2 swine influenza viruses with the 2009 pandemic H1N1 genes in pigs
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Reassortant H1 subtype of swine influenza viruses (SIVs) carrying genes from 2009 pandemic H1N1 virus (pH1N1) have been isolated from pigs worldwide. We isolated 3 genetically different H3N2 reassortant swine influenza viruses (SIVs) containing 3 or 5 genes of the 2009 pandemic H1N1 (pH1N1) virus from diseased pigs in Midwestern farms, the pathogenicity and transmissibility of these novel viruses remains unknown. Herein, we characterized these novel reassortant H3N2 viruses *in vitro* and in pigs using an endemic non-reassortant H3N2 SIV as a control. All these 3 novel reassortant H3N2 viruses grew to higher titers than the control endemic H3N2 SIV in canine, swine and human cell lines. In the pig study, all 3 novel reassortant viruses were able to replicate efficiently in lungs and transmitted to sentinel animals, similar to the control endemic H3N2 virus. The novel reassortant viruses with 3 genes (NP, M and NS) from pH1N1 were more transmissible when compared to the reassortant virus with 5 genes (PA, PB2, NP, M and NS) from pH1N1. Furthermore, concurrent molecular surveillance showed that the novel H3N2 virus with 3 genes from pH1N1 is continually isolated from swine herds and becomes a dominant H3N2 virus circulating in swine populations. All these results indicate that novel reassortant H3N2 virus may replace the endemic non-reassortant H3N2 SIV to be the dominant virus circulating in swine herds.

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Development of an equine ocular endothelial cell model to study equine herpesvirus myelitis (EHM)
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Despite the fact that Equine herpesvirus-1 (EHV-1) infection results in sporadic but devastating outbreaks of neurological disease in about 10% of infected horses, we have a rudimentary understanding of its pathogenesis. This is caused in part because of a lack of an adequate and ethically acceptable experimental model. EHV-1 infection of the vasculature of the eye, another secondary manifestation of EHV-1 infection, may be of interest because preliminary data suggests that the pathogenesis of ocular EHV-1 may be very similar to that of equine herpesvirus myelitis (EHM).
To determine the frequency of ocular EHV-1 following experimental infection with a neuropathogenic strain of EHV-1 and determine the potential value as a model for EHM, two experiments were designed. Experiment 1 employed classical fundus photography and fluorescent angiography during the acute phase (days 1-14 post infection) up until 90 days post infection to evaluate development and frequency of ocular lesions following infection. Experiment 2 compared wild type virus with a GFP expressing virus with the goal to localize the virus *in vivo* using a camera capable of GFP detection in the eye. Clinical signs, viral nasal shedding, viremia and SN titers were also determined following experimental infection in both experiments.
EHV-1 infection with a neuropathogenic strain of EHV-1 (Ab4) resulted in multifocal choroidal lesions in 90% of infected horses in experiment 1, and 50% of infected horses in experiment 2. Lesions appeared to be associated with the choroidal vasculature, while the retinal vasculature appeared to be unaffected. No lesions were detected during the acute phase of infection *in vivo*, however post mortem viral antigen could be detected during this period in the ocular vasculature and the spinal cord.

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In conclusion, this study showed that the frequency of ocular lesions induced by experimental infection with EHV-1 is reliably 50% or higher, and provides evidence that the route and pathogenesis of EHV-1 infection of the endothelia of the spinal cord and the eye is similar making the ocular model attractive for testing future vaccines or therapeutics in an immunologically relevant age group.

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Group C porcine Rotavirus subunit vaccine
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Group C Porcine Rotavirus is an emerging infection and is one of the leading causes of diarrhea and mortality in suckling piglets in the US. Currently, there are no vaccines available for controlling this disease. Traditional methods of virus isolation and growth in cell culture systems have not been successful. Commercially available Porcine Rotavirus vaccines include Group A Rotaviruses which differ significantly from Group C Rotaviruses so do not induce protection against Group C Rotaviruses in pigs. In this study, we evaluated the feasibility of a subunit Group C Rotavirus vaccine for the prevention of morbidity and mortality in suckling piglets.
Genes encoding for VP4, VP6 and NSP4 from a field strain of Group C porcine rotavirus were cloned into an E. coli expression vector. Recombinant proteins of VP4, VP6 and NSP4 were produced in E. coli and purified and formulated into a vaccine adjuvanted with Trigen. To evaluate this experimental vaccine's ability to confer protection, 48 sows were randomly divided into two groups, vaccinated (n=22) and control (n=26). The vaccinated group received two doses of vaccine 6 weeks and 3 weeks pre-farrowing and control group received placebo vaccination. After farrowing, the piglets were allowed to suckle and were monitored for 3 weeks. Mortality, scours and general body condition and growth was monitored and recorded. Blood serum was collected from the sows before and after vaccination as well as from piglets at 1 week of age to evaluate antibody titers. An ELISA was developed to monitor the serological response following vaccination.
Piglets born and that nursed on vaccinated sows had a 33% percent decrease in mortality and a significant reduction in scours incidence when compared to the control group. In addition, vaccinated sows and their suckling piglets had significantly higher antibody titers than non-vaccinated sows and suckling piglets as evaluated by ELISA ($p = 0.01$).
A subunit vaccine for Group C Porcine Rotavirus was developed that is able to induce protective immunity in suckling piglets.

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Genetic diversity of porcine circoviruses type 2 detected in pigs in Ukraine
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Purpose: Molecular epidemiology study of PCV-2-infection in Ukraine.
Methods:8 samples of viral DNA detected in different regions of Ukraine were analyzed. 421 bp rep gene fragments were sequenced and compared with sequences of viruses, belonging to genotypes 1 and 2. Phylogenetic analysis was carried out by Neighbor Joining algorithm under MEGA 5.0 software.
Results:8 samples of PCV-2 DNA were successfully amplified and purified after PCR. These fragments were used for sequencing. Corrected sequences distributed to four subgroups. Their diversity inside the group was up to 1.3 %, and between groups- 3-9 %. Philogenetic comparison of detected strains demonstrated their belonging to 1^{st} ($n = 5$) and 2^{nd} ($n = 3$) genotypes. 2^{nd}genotype belonging viruses are typically related to contaminants with North American origin, and else viruses belong to European genotype, and related with viruses, detected in Slovak republic, Germany, Poland and some others European countries.
Conclusions: Molecular diversity of PCV-2 population in Ukraine represents two viral lineages, circulating in pig farms. This should be used for development of successful prophylaxis measures.

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Characterization of the first complete genome sequence of the North American beaver (*Castor canadensis*) papillomavirus
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The papillomaviruses (PVs) comprise a large group of highly species-specific viruses that cause proliferations of the stratified squamous epithelium of skin or mucosa in a variety of animals. The previous report, for the first time, confirmed the papillomaviral etiology of cutaneous exophytic lesions in a North American beaver at the molecular level. In the current study we present the entire genomic sequence of Castor Canadensis papillomavirus (CcanPV1) and its analysis. The CcanPV1 genome of 7435 bp long is organized into the seven classical papillomaviral open reading frames (ORFs), encoding five early proteins (E6, E7, E1, E2, and E4) and two late capsid proteins (L2 and L1), and a non-coding region, identified between the end of L1 and the start of E6. CcanPV1 shows very stable placement in the topology between Kappa-PV and Mu-PV genera in trees inferred from different concatenations of sequences (e.g., E1+E2, L2+L1). CcanPV1 L1 nucleotide sequence has <60% identity to other PVs, suggesting that the virus represents a new genus.

VIRAL PATHOGENESIS

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Expression of type I interferon-induced antiviral state during experimental infection with low or high virulence bovine viral diarrhea virus in beef calves

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The objective of this study was to compare the mRNA expression of host genes involved in type-I interferon-induced antiviral state (IFN-α, IFN-β, Mx-1, PKR, OAS-1 and ISG-15), and pro-apoptosis (Caspase-3, -8, and -9), after experimental infection of beef calves with low or high virulence noncytopathic (ncp) bovine viral diarrhea virus (BVDV) strains. Thirty BVDV-naïve, clinically normal calves were randomly assigned to three groups. Calves were intranasally inoculated with low (LV; n= 10, strain SD-1) or high (HV; n= 10, strain 1373) virulence ncp BVDV or BVDV-free cell culture medium (Control, n= 10). Quantitative RT-PCR was used to determine the mRNA gene expression of type-I interferon-induced antiviral state and pro-apoptosis markers in tracheo-bronchial lymph nodes and spleen 5 days after infection. Interferon-α and -β mRNA levels were up-regulated in tracheo-bronchial lymph nodes ($P<0.05$) in the HV group, but not in the LV group, compared with the control group. There was an up-regulation of type I interferon-induced genes in spleen and tracheo-bronchial lymph nodes of HV and LV groups, compared with the control group ($P<0.01$). mRNA levels of OAS-1 and ISG-15 were significantly higher in LV than HV calves ($P<0.05$). A significant up-regulation of caspase-8 and -9 was observed in tracheo-bronchial lymph nodes in the LV group ($P=0.01$), but not in the HV group. In conclusion, experimental infection with high or low virulence BVDV strains induced a significant expression of the type I interferon-induced antiviral state in beef calves. There was a differential expression of some interferon-induced genes (OAS-1 and ISG-15) and pro-apoptosis markers based on BVDV virulence and genotype.

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Differential expression of pro-inflammatory and anti-inflammatory cytokines during experimental infection with low or high virulence bovine viral diarrhea virus in beef calves

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The objective of this study was to compare the mRNA expression of cytokines involved in pro-inflammatory (TNF-α, IL-1β, IFN-γ, IL-2, IL-12, IL-15), and anti-inflammatory (IL-4, IL-10, TGF-β) responses after experimental infection of calves with low or high virulence noncytopathic (ncp) bovine viral diarrhea virus (BVDV) strains. Thirty BVDV-naïve, clinically normal beef calves (seronegative to BVDV) were randomly assigned to one of three groups. Calves were intranasally inoculated with low (LV; n= 10, SD-1) or high (HV; n= 10, 1373) virulence ncp BVDV or with BVDV-free cell culture medium (Control, n= 10). Calves were euthanized on day 5 post-inoculation and tissue samples of tracheo-bronchial lymph nodes and spleen were collected for quantitative-RT-PCR analysis to determine the mRNA level of the target genes. mRNA levels of pro-inflammatory (TNF-α, IL-1β, IL-2, IFN-γ) and anti-inflammatory (IL-4 and IL-10) cytokines were significantly up-regulated in tracheo-bronchial lymph nodes of HV group, but not in LV group, compared to the control group ($P < 0.05$). The IL-12 mRNA level was up-regulated in tracheo-bronchial lymph nodes of both LV and HV groups, compared with the control group ($P \leq 0.05$). A significant up-regulation of IL-15 mRNA was observed in tracheo-bronchial lymph nodes for LV calves ($P < 0.002$), but not for HV calves. Experimental inoculation with BVDV-2 1373 stimulated a significant mRNA expression of both pro-inflammatory and anti-inflammatory cytokines. However, inoculation with BVDV-1 SD-1 only resulted in up-regulation of IL-12 and IL-15 mRNA, which are associated with activation of macrophages and NK cells during the innate immune response.

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PCR-screening of chlamydia and viral contamination of bovine semen in Ukraine

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Purpose: Screening of bull semen in order to find its contamination with Chlamydia, viruses of vira ldiarrhoea (VD), infectious rhinotracheitis (IRT), and rotaviral infection (RI) is the essential step to define the role of semen in transmission of these infections and their outbreaks in farms of Southern and Eastern Ukraine.
Methods: Screening was conducted with conventional PCR technique using primers CHOMP, BVDV, IRTV, rotab. In total 252 bull semen samples were investigated. Samples were taken from bulls of different breeds in 8 farms with high burden of respiratory, reproductive and gastrointestinal infections in Kharkiv, Lugansk, Donetsk, Dnipropetrovsk, Odessa, and Mykolayiv regions. Vaginal swabs and pathological material from misbirths were investigated in separate cases.
Results: Chlamydial DNA was found in 15 semen samples taken in 2 farms. In one of these farms mixed infection of infectious rhinotracheitis and chlamydiosis was found. These cases were accompanied by abortions, birth of nonviable calves, and affection of joints during the first year of life. The infectious agent was detected both in semen and in clinical material and identified as *Chlamydophila abortus*. In semen samples from another farm chlamydial DNA was found only. Bovine diarrhoea virus was found in 22 % of investigated samples, and in most cases these samples were also contaminated with RNA of rotavirus. DNA of herpesvirus was detected in 12 % of samples and was further differentiated as IRT virus type I. In farms, where semen infected with IRT virus was used, disorders of reproduction system accompanied with decreasing of percentage of calf birth caused by abortions and fertility were observed. Investigations of vaginal swabs and pathological material from misbirths revealed also presence of infectious rhinotracheitis virus DNA in 14 % of cases.
Conclusions: The results of PCR screening showed very high percentage of bull semen contamination (33 % of all samples). PCR proved to be one the most rapid and sensitive instrument for large-scale screening.

VIRAL PATHOGENESIS

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Of Men, Pigs, Birds and···Flu

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Influenza A viruses (IAVs) belong to the family *Orthomyxoviridae* and represent major pathogens of both humans and animals. Intricate and complex animal reservoirs have made influenza viruses the paradigm of emerging diseases. Pigs have been historically considered a “mixing vessel” for the generation of novel influenza viruses. Pigs are susceptible to human influenza viruses; however, and perhaps unlike humans, they appear susceptible to a wide range of avian influenza viruses. Pigs were undoubtedly involved in the genesis of the 2009 pH1N1. Since then, several reassortants between pH1N1 and circulating influenza A viruses have been isolated from pigs in several countries, raising great concerns about the potential acquisition of virulence markers by the pH1N1 virus upon reassortment with other strains in the swine host. In addition, the emergence influenza strains in birds with the ability infect humans and pigs and the lack of adequate prevention strategies, other than enhanced biosecurity and surveillance, have made influenza viruses an unstoppable moving target. In this presentation, we will discuss aspects of transmission of influenza among different animal species, molecular features associated with host switching and the potential use of live attenuated influenza vaccines as a tool to prevent the spread of these viruses.

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