



PROGRAM and PROCEEDINGS

**of the 91st Annual Meeting
December 5, 6 and 7, 2010**

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

Robert P. Ellis, Executive Editor

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

**The 91st Annual Meeting of the
CRWAD is dedicated to**

Dr. Samuel K. Maheswaran

Proceedings Distributed by CRWAD

CRWAD 91st ANNUAL MEETING-2010

December 5 – 7, 2010

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

CRWAD Researchers Reception - Welcome all attendees. Casual Wear

Sunday, December 5, 6-8 PM – Grand Ballroom Salon III - 7th Floor

Introduction of CRWAD Officers and Dedicatee

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

11:45 AM - 12:30 PM Tuesday, December 7

Dedication of the 2010 meeting to Dr. Sam K. Maheswaran

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) - Friday, Dec. 3 - Tuesday, Dec. 7

Marriott Hotel	Monday AM 8:00 - 11:30 Room Abstract Nos.	Monday PM 1:30 - 4:30 Room Abstracts Nos.	Tuesday AM 8:00 - 11:30 Room Abstracts Nos.
Bacterial Pathogenesis	Avenue Ballroom 001 - 011	Avenue Ballroom 012 - 022	
Biosafety and Biosecurity		Denver/Houston 023 - 030	
Companion Animal Epidemiology			Avenue Ballroom 031 - 042
Epidemiology and Animal Health Economics	Salons A/B/C/D 043 - 051	Salons A/B/C/D 052 - 062	Salons A/B/C/D 063 - 074
Food and Environmental Safety	Salon E 075 - 084	Salon E 085 - 095	Salon E 096 - 101
Gastroenteric Diseases	Michigan/Michigan State 102 - 111	Michigan/Michigan State 112 - 116	
Immunology	Salons F/G/H 117 - 127	Salons F/G/H 128 - 136	Salons F/G/H 137 - 144
Respiratory Diseases	Indiana/Iowa 145 - 157	Indiana/Iowa 158 - 166	
Vector-Borne and Parasitic Diseases	Denver/Houston 167 - 176		
Viral Pathogenesis	Los Angeles/Miami 177 - 188	Los Angeles/Miami 189 - 199	Los Angeles/Miami 200 - 203
Posters*	Grand Ballroom Salon III Sun. 6:30 - 8 PM	Grand Ballroom Salon III Mon. 5 - 6:30 PM	

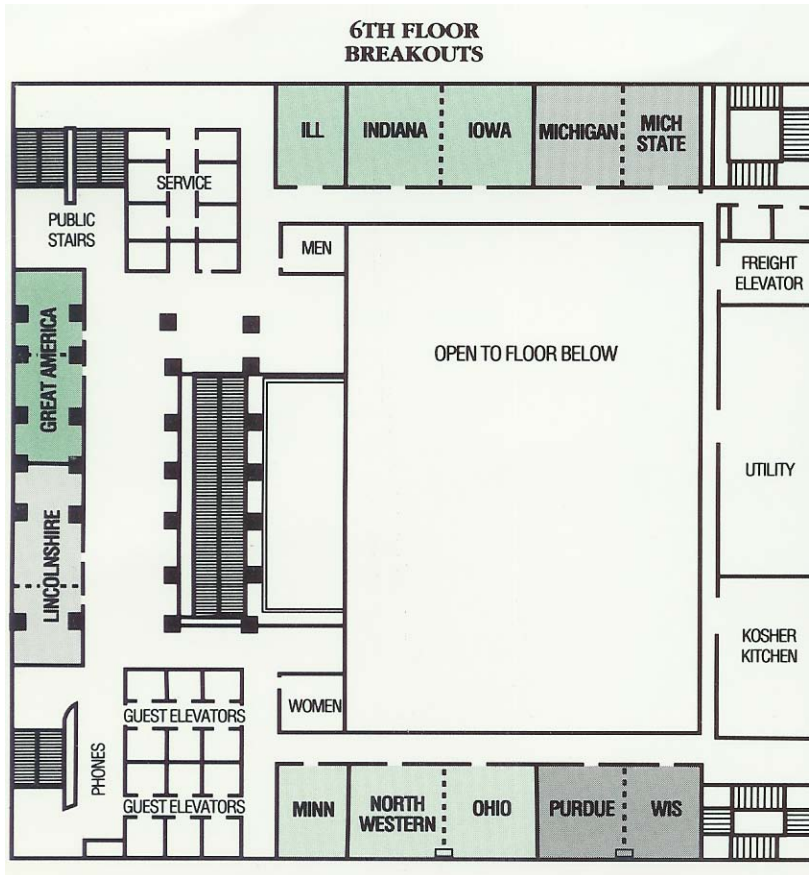
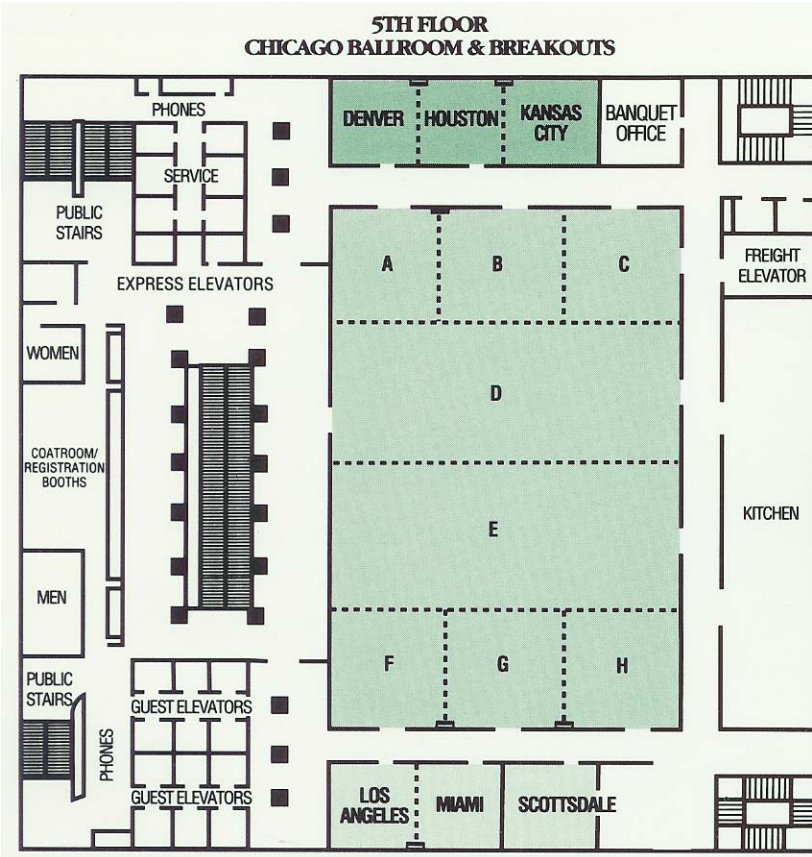
***SUNDAY POSTER PRESENTERS:** Poster boards will be available for poster assembly by 4 PM Sunday. Posters for the Bacterial Pathogenesis, Biosafety and Biosecurity, Companion Animal Epidemiology, Epidemiology and Animal Health Economics, Food and Environmental Safety, and Gastroenteric Diseases Sections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

***MONDAY POSTER PRESENTERS:** Poster boards will be available for poster assembly by noon Monday. Posters for the 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 wear their name badge.

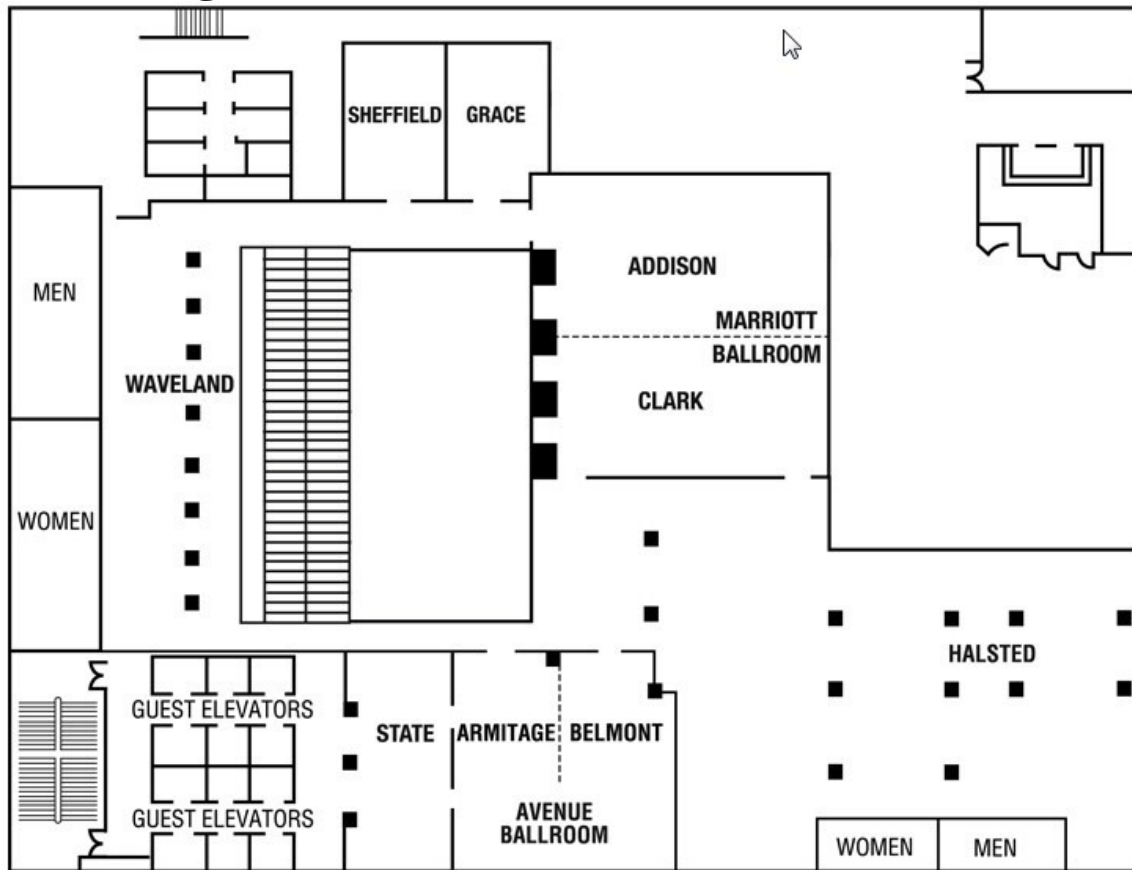
Poster Presenters must be with their competition entry posters for possible judge interviews and must wear their name badge during their presentation.

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

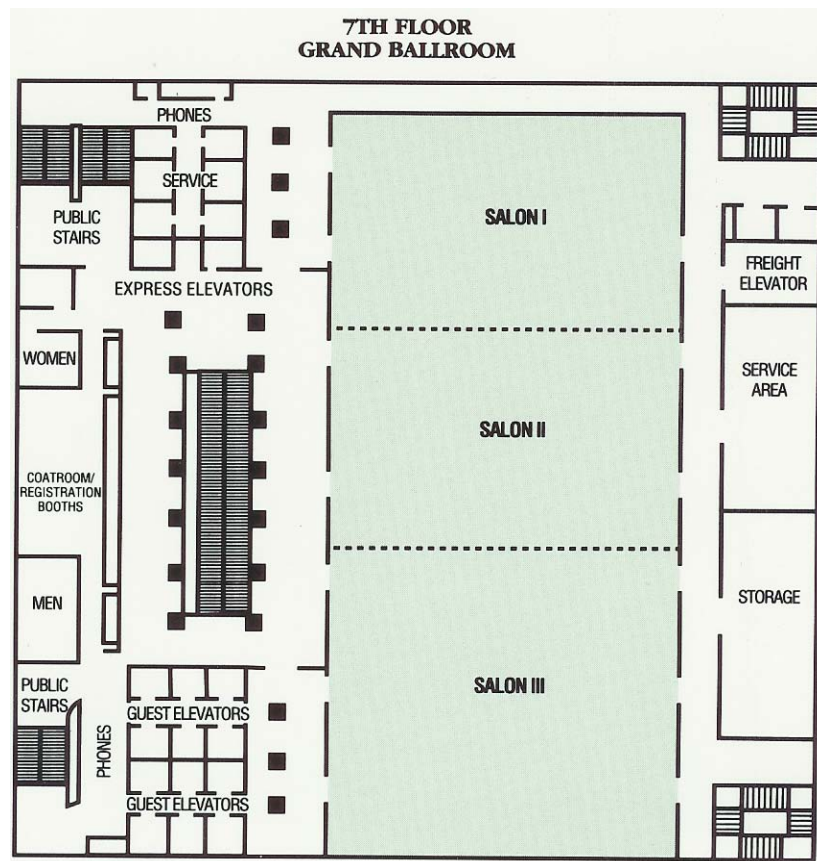
Chicago Marriott, Floor Plan - 5th and 6th Floors



Chicago Marriott Floor Plan – 4th and 7th Floors



Avenue Ballroom on 4th Floor



CRWAD 2010 Table Top Exhibitors

**Chicago Marriott, Downtown Magnificent Mile
5th Floor Foyer**

Saturday, December 4 - Tuesday, December 7

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CRWAD

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CRWAD

Meeting and Organization Information

The Conference of Research Workers in Animal Diseases (CRWAD) was founded in Chicago in 1920. The CRWAD Annual Meeting is held on a Sunday, Monday and Tuesday of December, and consists of oral and poster presentations. The presentations are arranged into the following 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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2010 Officers

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Vice President - Laura Hungerford
Executive Director - Robert P. Ellis

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Rodney A. Moxley (2007 - 2011)
David A. Benfield (2008 - 2012)
Roman R. Ganta (2009 – 2013)

Recent Past Presidents

Bill Stich - 2009	Lynn A. Joens - 2007
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Franklin A. Ahrens - 2002	M. D. Salman - 1999
Leon N. D. Potgieter - 2000	Bert E. Stromberg - 1997
Donald G. Simmons - 1998	Bradford B. Smith - 1995
Patricia E. Shewen - 1996	Lawrence H. Arp - 1993
Ronald D. Schultz - 1994	Robert M. Corwin - 1991
Richard F. Ross - 1992	William C. Wagner - 1989
Lynette B. Corbeil - 1990	

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 2010 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		

2010 CRWAD Dedicatee – Samuel K. Maheswaran



Dr. Sam Maheswaran is Professor Emeritus of Microbiology at the College of Veterinary Medicine, University of Minnesota. He received his primary, middle, and senior high school education at a Methodist Missionary Institution in Jaffna, Ceylon (now called Sri Lanka). He earned his Bachelor of Veterinary Science degree from the University of Ceylon in 1960, and MS (1966) and PhD (1967) degrees from the University of Minnesota.

After a brief stint as a Lecturer at the University of Ceylon, in 1968 Sam accepted a Post-doctoral fellowship at the Department of Microbiology, University of Manitoba Medical School and worked under the tutelage of Professor Gordon Wiseman. After a short 14 months, Sam moved back to the University of Minnesota in 1969 as an Instructor in the Department of Veterinary Microbiology. In 1971, he was appointed as an Assistant Professor in the same department. Indeed, he together

with another individual was the first person of color to be appointed as Assistant Professor in the College of Veterinary Medicine, University of Minnesota. Sam has enjoyed a distinguished career at the University of Minnesota for forty years.

Together with Russ Bey, Sam taught an undergraduate General Microbiology and Immunology course in the Twin Cities campus for over 30 years. This course is the most popular undergraduate Microbiology course which now has a heavy demand in the campus to get into the course and has an enrollment of over 440 students per year. Sam has also participated in teaching graduate courses at the Department of Veterinary Pathobiology.

He spent his research career at the University of Minnesota studying the biology of bacterial species belonging to the family Pasteurellaceae which includes a number of pathogens which cause respiratory diseases in cattle, pigs, and turkeys. Most of his research endeavors were centered on *Mannheimia haemolytica* which is the causative agent of bovine respiratory disease (BRD). In 1980, his laboratory pioneered the use of a cell culture medium namely, RPMI 1640 to grow log phase *M. haemolytica* which produced copious amounts of cytotoxin when grown in this medium. Some of the other landmark contributions on *M. haemolytica* from his laboratory are: in 1984, he coined the name “leukotoxin” for the exotoxin cytotoxin; used a well characterized reproducible experimental model of the disease in calves and showed that leukotoxin was the most important contributor to the lung pathology in BRD; his lab was the first to purify LPS free leukotoxin using preparative SDS-PAGE; showed that leukotoxin was a protective antigen and vaccine preparations containing leukotoxin were far superior in inducing

2010 CRWAD Dedicatee – Samuel K. Maheswaran

protective immunity than vaccines without the leukotoxin; demonstrated a correlation between the pulmonary expression of IL-8 and acute pneumonic pasteurellosis; and together with other investigators identified CD18 as the receptor for leukotoxin on bovine leukocytes. In the 1970s, his laboratory was the first to isolate *Actinobacillus pleuropneumoniae* from the pneumonic lungs of dead pigs in the State of Minnesota and developed a capsular typing technique. And finally, his laboratory pioneered the use of a modified live vaccine administered in the drinking water to protect commercial turkeys against avian cholera.

He has published over 100 refereed journal articles, and over 100 abstracts and proceedings during his research career at Minnesota. Sam has also delivered keynote addresses at several national and international conferences. Sam has also held administrative responsibilities at the University of Minnesota. He served as the Vice Chair and then Acting Chair of the Department of Veterinary Pathobiology for a total of almost six years. He serves in the Editorial Board of two journals and had served in the study section of the USDA-NRI competitive grants for four years. During his tenure at Minnesota he has trained 23 graduate and postdoctoral students and numerous undergraduate researchers.

Sam retired in September of 2009, but still goes to work for several hours five days of the week. And he also spends time consulting and part time teaching. His hobbies are gardening, fishing, and he is an astute student of American football, college hockey, and college basketball.

**2010 CRWAD - Keynote Speaker - Immunology Section
AAVI Distinguished Veterinary Immunologist Award**

**Dr. Frank Blecha – College of Veterinary Medicine, Kansas State University,
Manhattan KS**

Abstract No. 128 - Title: Interplay of Antimicrobial Peptides and Interferons in PRRSV Infections.

Dr. Frank Blecha is a University Distinguished Professor at Kansas State University in Manhattan, Kansas where he is the Associate Dean for Research and Head of the Department of Anatomy and Physiology in the College of Veterinary Medicine. He earned his bachelor's and master's degrees from the University of Idaho and his doctorate from Washington State University in Pullman, Washington. His research is focused on the interrelationship of immunology and physiology, and regulatory mechanisms involved in innate immunity. He has authored 139 refereed publications, 24 book chapters, more than 200 abstracts, and four U.S. patents.

Dr. Blecha's studies on the immune system have been supported by grants from the NIH, the U.S. Department of Agriculture, and the American Heart Association. His expertise has been recognized by invitations to serve on several USDA and NIH study sections and advisory panels. He has held leadership positions in a number of national and international organizations, including the American Association of Immunologists, and the American Association of Veterinary Immunologists. He serves on several journal Editorial Boards in his areas of interest.

Dr. Blecha's primary research interest is in immunology and host-pathogen interactions. A current focus is on the isolation and characterization of antimicrobial peptides and antiviral cytokines. Although most studies on antimicrobial peptides usually have focused on bacterial and fungal killing, these host defense peptides have antiviral properties and other nonmicrobicidal functions that mitigate viral infection. A current major interest in Dr. Blecha's lab is focused on understanding the interactions between antimicrobial peptides, antiviral cytokines, viruses, and pattern recognition receptors.

**2010 CRWAD - Keynote Speaker for the Viral Pathogenesis Section
And Distinguished Veterinary Microbiologist**

Frederick A. Murphy

University of Texas Medical Branch
Galveston, TX

Abstract No. 201 - Title: New and Emerging Diseases: A Very Personal Perspective

Frederick A. Murphy is Professor, Department of Pathology, University of Texas Medical Branch, Galveston. He holds a BS and DVM from Cornell University and a PhD from the University of California, Davis. Formerly he was Dean and Distinguished Professor, School of Veterinary Medicine, and Distinguished Professor, School of Medicine, University of California, Davis. Before that he served as Director of the Division of Viral and Rickettsial Diseases and then Director of the National Center for Infectious Diseases, Centers for Disease Control, Atlanta. He is a member of the Institute of Medicine of the U.S. National Academy of Sciences and is a member of the German National Academy of Sciences. His honors include an honorary Doctor of Medicine and Surgery, University of Turku, Turku, Finland, an honorary Doctor of Science, University of Guelph, Ontario, Canada, the Presidential Rank Award of the U.S. Government, and the PennVet World Leadership Award from the University of Pennsylvania. At UTMB he is a member of the Institute for Human Infections and Immunity, The Center for Biodefense and Emerging Infectious Diseases, The Galveston National Laboratory, and the McLaughlin Endowment for Infection and Immunity.

Dr. Murphy's professional interests include the pathology and epidemiology of highly pathogenic viruses/viral diseases: (1) Rabies: long running studies leading to the identification of more than 25 viruses as members of the virus family Rhabdoviridae, identification and characterization of the first rabies-like viruses, and major studies of rabies pathogenesis in experimental animals, including the initial descriptions of infection events in salivary glands and in muscle. (2) Arboviruses: long running studies of alphaviruses, flaviviruses and bunyaviruses with the initial proposal for the establishment and naming of the virus family Bunyaviridae, and characterization of "reo-like" viruses culminating in the establishment and naming of the virus genus Orbivirus. (3) Viral hemorrhagic fevers: long running studies leading to the initial discovery of Marburg and Ebola viruses, and characterization of several other hemorrhagic fever viruses, culminating in the establishment and naming of the virus families Arenaviridae (e.g., Lassa and Machupo viruses) and Filoviridae (Marburg and Ebola viruses), and elucidation of the pathology and pathogenesis of the diseases in man, monkeys, hamsters and guinea pigs caused by these exceptionally virulent agents. (4) Viral encephalitides: long running studies of the pathogenesis of neurotropic viruses in experimental animals, including alphaviruses, flaviviruses, bunyaviruses, enteroviruses, paramyxoviruses, herpesviruses, and others.

Dr. Murphy has been a leader in advancing the concept of "new and emerging infectious diseases" and "new and emerging zoonoses," which has reinvigorated and reenergized the infectious disease research sciences. Most recently his interests have included the threat posed by bioterrorism.

In Memoriam – George Calvin Shelton, 1923 - 2010



Few veterinarians have faced a decision of the sort that confronted George Shelton in 1973 when, on the same day, he was offered the opportunity to serve as dean of two veterinary schools. He chose Texas A&M, his alma mater, over the University of Missouri where he had taught parasitology for twenty-four years and been associate dean for academic affairs for seven. Following fifteen years (1973-1988) as dean at Texas A&M, he and his wife returned to Missouri for retirement.

A native Texan, Shelton was a World War II veteran. Before embarking on military service he completed the A.S. degree at Tarleton State University at Stephenville, Texas, a school near the small dairy farm where he grew up. As a 19-year-old radioman and gunner on B-25 bombers, he ran 50-odd combat missions in the Southwest Pacific. Following this harrowing service he

returned to school to study veterinary medicine. Like many others, the \$50./month stipend from the GI Bill of Rights enabled him to pursue higher education and he took full advantage of it.

Following graduation in 1948, Shelton established a private veterinary practice in Moniteau County, Missouri; but he soon developed undulant fever and left practice. He was invited to join the nascent veterinary faculty at the University of Missouri-Columbia. Dean A. H. Groth permitted Shelton to take leave for further study and he went to Auburn University where he earned his M. S. degree under direction of the distinguished veterinary parasitologist, Wilford S. Bailey. This period of study was sponsored, in part, by a scholarship from the R. C. Cola Bottling Company. He and Bailey published papers that dealt with naturally occurring and experimentally induced giardiasis in the chinchilla as well as experimental tapeworm infection in that host.

Shelton taught parasitology to veterinary students very successfully at Missouri and published a number of papers on parasitism in sheep, cattle, swine, and poultry. He eventually took leave-of-absence to go to the University of Minnesota where he earned his Ph.D. under direction of Henry J. Griffiths in 1965. They published studies involving *Oesophagostomum columbianum* infections in lambs. Shelton continued work with ruminant parasites and contributed to the scientific literature on anthelmintics and other parasiticides, including drugs aimed at protozoan parasites of poultry. A member of many professional organizations, Shelton was active in the Conference of Research Workers in Animal Diseases and the American Association of Veterinary Parasitologists, serving the latter organization as president in 1967-68.

Shelton was tapped for administrative service at Missouri by Dean B. W. Kingrey, first as an academic department chair and assistant dean in 1965. He became Associate Dean for Academic Affairs the following year and simultaneously continued as department chair until 1969. He was

In Memoriam – George Calvin Shelton, 1923 - 2010

Associate Dean from 1966 to 1973. His 15-year stint as Dean of Veterinary Medicine at Texas A&M was exceeded in length only by its first dean, Mark Francis (1916-1936) and Shelton's immediate predecessor, Alvin A. Price (1957-1973).

In retirement Shelton published *Treetop Airmen*, a historical novel based on the experiences of a strafe combat crew that flew in the Southwest Pacific in 1944-1945. Anyone interested in World War II history would likely find this novel, which is highly autobiographical, of interest. At the time of his death Shelton was working on a second book, a historical work concerning interactions between Mark Francis in Texas and J. W. Connaway in Missouri. Shelton aimed to capture the essence of professional contributions by these two giants of the veterinary profession while relating details of their personal lives. He focused especially on the importance of their cooperative work on Texas cattle fever and contagious abortion (brucellosis) and emphasized the visionary character of their professional thinking.

Sidney A. Ewing
Oklahoma State University

DR. JOHANNES 'HANS' STORZ – In Memoriam 1931 - 2010

Johannes "Hans" Storz, 79, died Friday, Oct. 1, 2010. A service to celebrate his life will be held at the Ryssby Church, 9000 N. 63rd St., Longmont, Colorado, at 4 p.m. Monday, Nov. 1. Johannes Storz was born April 29, 1931, in Hardt, Germany. He was raised in very humble circumstances on the family farm, the eighth of 10 children. While a youngster, Johannes' oldest sister recognized his potential and encouraged his parents to allow him to attend high school. Subsequently, Johannes studied veterinary medicine at the universities in Hannover and Munich and performed his doctoral work at the veterinary research institute in Tuebingen, Germany.

In 1958, Johannes Storz ventured to the United States as a Fulbright Scholar and completed his Ph.D. at the University of California, Davis. He then accepted a position as an assistant professor at Utah State University in Logan, Utah, where he carried out work on agents such as the chlamydia and corona viruses that cause infections in cattle. In 1965, Johannes moved his young family to Fort Collins, Colorado, where he taught veterinary virology, microbiology and parasitology at Colorado State University for 17 years. While at CSU, Johannes Storz was awarded the Alexander von Humboldt Prize for his groundbreaking work on chlamydia. In 1982, Johannes Storz moved to Baton Rouge to become the department head of Veterinary Microbiology and Parasitology at Louisiana State University. During his 19 years at LSU, Johannes Storz developed a vaccine for a bovine gastro-intestinal disease known as "shipping fever" and received an honorary doctorate from the University of Zurich. Throughout his career, Johannes enjoyed collaborating with scientists from around the world, particularly with colleagues at the Justus Liebig University in Giessen, Germany. He mentored many students and fellows and was an outstanding teacher, demanding excellence while conveying his passion for animals, science and learning. Hans retired in 2002 and moved to Longmont, where he became an active member of the community. After his retirement his colleagues in the Conference of Research Workers in Animal Diseases elected him to be the dedicatee of the 2002 CRWAD annual meeting. This is the highest honor awarded by CRWAD.

All would agree that Hans was lucky in love, convincing Hannelore Roeber, a charming research associate he met in Tuebingen, to come to America and to marry him in 1959. The family life he fashioned with Hannelore served as a source of great strength for him, and he always credited Hannelore with helping him achieve his full potential. Hans Storz had a commanding presence in part because of his solid physical stature, but even more so because of his exuberance for life. He had a beaming smile that many people saw at social functions, family reunions, departmental gatherings or community events. His smile was never greater than when he approached his grandchildren with a twinkle in his eye and a bag of gummi bears in his hand. However, his most defining feature was a sharp and insightful mind adept at discerning facts and divining character. We are blessed that when his heart gave way, Hans was fully engaged in life until his last sentence - in which he was recalling fondly the arc of his life in the company of his daughter and the family of his esteemed scientific mentor. He found peace Friday, Oct. 1, 2010, in Hamburg, Germany, with his three children by his side. Johannes Storz was preceded in death by his wife, Hannelore. He is survived by four siblings; his eldest daughter, Gisela Therese Storz, her husband, Carl Wu, and their three children, Ella, Toby and Felix; his son, J. Peter K. Storz, his wife, Carolyn Smith Storz, and their four children, Olivia, Sophie, Natalia and Alexander; his youngest daughter, Heidi Ella Storz, her husband, Roque Ramirez, and their two children, Roberto and Marco; Pat Blankenship, his loving companion of later years; and many lifelong friends. In lieu of flowers, memorial contributions can be made in Johannes' name to the "Friends of the Longmont Senior Center", P.O. Box 313, Longmont, CO 80502.

PROGRAM



CRWAD THANKS THE FOLLOWING 2010 SPONSORS

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December 5-7

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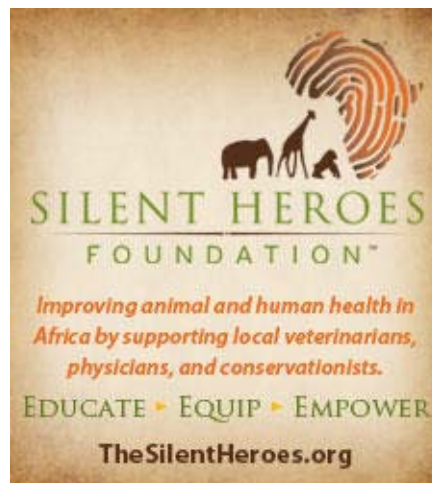
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The CRWAD Conference is supported by the National Research Initiative (NIFA) of the USDA Cooperative State Research, Education and Economics National Institute of Food and Agriculture Award No. 2010-65119-20597 .

2010 CRWAD Keynote Speakers and Titles

Bacterial Pathogenesis Section – Dr. Larry S. Schlesinger

Director, Division of Infectious Diseases and the Center for Microbial Interface Biology
The Ohio State University, Columbus, OH

Monday, December 6, 10:45 AM - Avenue Ballroom, 4th Floor

No. 011 - Title - Interplay of *M. tuberculosis* and macrophages in the human respiratory system.

Biosafety and Biosecurity Section – Dr. Paul S. Morley

Director of Biosecurity, James L. Voss Veterinary Teaching Hospital, Colorado State University, Fort Collins, CO

Monday, December 6, 3:00 PM - Denver/Houston Room, 5th Floor

No. 028 - Title - Beyond normal science: paradigm shifts in veterinary infection control.

Epidemiology & Animal Health Economics, and Food & Environmental Safety Sections – Dr. H. Scott Hurd

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

Monday, December 6, 8:00 AM - Salons E, 5th Floor

No. 075 - Title - Food Systems Veterinary Medicine for the 21st Century

Gastroenteric Diseases Section – Dr. Chobi DebRoy

E. coli Reference Center, The Pennsylvania State University, University Park, PA

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

No. 105 - Title - Unraveling the mysteries of O-antigens in *Escherichia coli*.

Immunology Section – Distinguished Veterinary Immunologist – Dr. Frank Blecha

College of Veterinary Medicine, Kansas State University, Manhattan, KS

Monday, December 6, 1:30 PM - Salons F/G/H, 5th Floor

No. 128 - Title - Interplay of Antimicrobial Peptides and Interferons in PRRSV Infections

Respiratory Diseases - Dr. Tanja Opriessnig

Vet. Diagnostic and Production Animal Medicine, CVM, Iowa State University, Ames, Iowa

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

No. 166 - Title - Polymicrobial respiratory disease in pigs.

Vector-Borne and Parasitic Diseases – Dr. Katherine M. Kocan

Oklahoma State University Regents Professor and the Walter R. Sitlington Endowed Chair in Food Animal Research, Stillwater, OK

Monday, December 6, 10:00 AM - Denver/Houston Room, 5th Floor

No. 173 - Title - Bovine anaplasmosis: an overview of current challenges.

Viral Pathogenesis Section – Distinguished Veterinary Microbiologist - Dr. Frederick A. Murphy

University of Texas Medical Branch, Galveston, TX

Tuesday, December 7, 8:15 AM - Los Angeles/Miami/Scottsdale, 5th Floor

No. 201 - Title – New and Emerging Diseases: A Very Personal Perspective

2010 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. 5, 10 AM - 5:30 PM
Monday, Dec. 6, 7:00 AM - Noon, 2 - 5 PM
Tuesday, Dec. 7, 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. 5, 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. 6 - 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.

American Association of Veterinary Immunologists (AAVI)

Sunday, Dec. 5, **Board Meeting**
8 AM - 12 PM - Los Angeles Room - 5th Floor
Monday, Dec. 6, **Business Meeting and Luncheon**
11:30 AM - 1PM - Buca di Beppo Restaurant
For more information contact Gina Pighetti

ACVM/AAVI Symposium - Title: Emerging and Re-Emerging Zoonotic Pathogens

Sunday, Dec. 5 - 1:30 to 5 PM, Chicago Ballroom Salons D/E - 5th Floor
For more information about the meeting contact Christopher Chase, South Dakota State University, or Paul Coussens, Michigan State University.

American College of Veterinary Microbiologists (ACVM)

Examination - Denver/Houston Room - 6th Floor
Friday, Dec. 3, 12 PM - 8 PM
Saturday, Dec. 4, 8 AM - 9 PM

Sunday, Dec. 5, - Denver/Houston Room - 6th Floor
8 AM - 10 AM - Examination Committee Meeting
10 AM - 12 PM - Board of Governors Meeting. Attendance is by invitation only.
For more information contact Amelia Woolums.

Animal Health Research Reviews (AHRR) Board Meeting

Tuesday, Dec. 7, 7 - 9:30 AM – Great America Room - 6th Floor
Section Editors and Editorial Board joint meeting.
For more information contact Carlton Gyles, Editor in Chief

AVEPM Schwabe Symposium - Methodologies in Epidemiological Research

A Symposium Honoring the Legacy of Dr. Preben W. Willeberg
(Association for Veterinary Epidemiology and Preventive Medicine)
Sunday, Dec. 5, 11:30 PM - 5 PM, Chicago Ballroom Salons F/G/H - 5th Floor
For more information contact Paul Morley

2010 CRWAD AND SATELLITE MEETINGS

(Alphabetically listed)

AVEPM Business Meeting – Buffet Luncheon - Members only **(Association for Veterinary Epidemiology and Preventive Medicine)**

Monday, Dec. 6, 11:30 AM - Great America Room - 6th Floor

For more information contact Jan Sargeant or Morgan Scott
A Symposium Honoring the Legacy of Dr. Preben W. Willeberg
Formal presentation will be during CRWAD Business Meeting
Tues. Dec. 7, 11:45 AM - 12:30 AM - Chicago Ballroom A/B/C/D - 5th Floor

Brucellosis Research Group Meeting

Saturday, Dec. 4, 7 AM - 8:45 AM - Registration - Salons DA - 5th Floor

Saturday, Dec. 4, 8 AM - 5 PM - Salons DA - 5th Floor

Sunday, Dec. 5, 8:15 AM - 12 PM - Salons DA - 5th Floor

Attendance is by invitation only.

For more information contact Sue Hagius, Secretary/Treasurer

Boehringer Ingelheim Workshop

Thursday, Dec. 2, 12:00PM – 10:00PM - Salon ABC Room 5th Floor

Friday, Dec. 3, 6:00 AM - 12 PM - Salon ABC Room 5th Floor

For more information contact Leigh Ann Cleaver

CRWAD Council Meeting

Saturday, Dec. 4, 5:30 PM - 9 PM - Great America Room - 6th Floor

CRWAD Business Meeting

Tuesday, Dec. 7, 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 Presentations.

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

CRWAD Sponsorship Committee Meeting

Saturday, Dec. 4, 4:00 – 5:30 PM, Minnesota Room – 6th Floor

Distinguished Veterinary Immunologist Lecture by Dr. Frank Blecha

Kansas State University, Manhattan, KS

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

Title - Interplay of Antimicrobial Peptides and Interferons in PRRSV Infections

Distinguished Veterinary Microbiologist Lecture to honor Dr. Frederick A. Murphy

University of Texas Medical Branch, Galveston, TX

Tuesday, December 7, 10:15 AM - Los Angeles/Miami/Scottsdale, 5th Floor

Title - New and Emerging Diseases: A Very Personal Perspective

Elsevier Meeting – Editorial Board of Preventive Veterinary Medicine

7:30 AM - 9 AM - Kansas City Room, 5th floor

Exhibitors - (Table Top) Saturday - Tuesday, Dec. 4-7, 5th Floor Foyer

8 AM – 6 PM - 5th Floor Foyer (close Tuesday, Dec. 7, 10 AM)

Elsevier BV

Eppendorf North America

Qiagen, Inc.

Sarstedt, Inc.

Seppic, Inc.

Tetracore, Inc.

2010 CRWAD AND SATELLITE MEETINGS (Alphabetically listed)

Integrated Special Emphasis Project

Minimizing Antibiotic Resistance Transmission throughout the Food Chain
Saturday, Dec. 4, 1:00 PM - 5:00 PM, Northwestern/Ohio Room – 6th Floor
Sunday, Dec. 5, 8:00 - 11:00 AM, Northwestern/Ohio Room – 6th Floor

Attendance is by invitation only.

For more information contact H. Morgan Scott, Kansas State University: 785-532-4602

International PRRS Symposium (IPRRSS)

Friday, December 3, Salons E-F-G-H, 5th Floor - Registration required for attendance.

1:00-2:30 PM - Business meeting Multistate Research Project NC-229

2:00 PM Poster Boards available for poster assembly - Salons F/G/H

2:30-4:00 PM - PRRSV Elimination Workshop/USDA CAP-2 meeting

4:00 PM - Opening Session, Keynote Presentation by Dr. Ralph Baric

5:15PM - 7 PM - IPRRSS Reception/Mixer (cash bar)

5:15 - 7 PM - First Poster Session

Saturday, December 4, Salons E-F-G-H, 5th Floor - Registration required for attendance.

8 AM - 5 PM, Oral Presentations

9:40 AM - Second Poster Session

2:40 PM - Break

For more information contact Joan Lunney, Lisa J. Becton (at NPB), Bob Rowland or X.J. Meng.

NC-1041 Enteric Diseases of Swine and Cattle Meeting

Saturday, Dec. 4, 8 AM - 5 PM – Kansas City Room - 5th Floor

Sunday, Dec. 5, 8 AM - 12 PM - Kansas City Room - 5th Floor

Attendance is by invitation only.

For more information contact Linda Mansfield. (mansfie4@cvm.msu.edu)

PRRSV Diversity (CAP-2 Sub-Project)

8:00 AM to 12:00 PM - Great America I/II Rooms – 6th Floor

For more information contact Dr. Fernando Osorio fosorio@unl.edu

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

2010 CRWAD AND SATELLITE MEETINGS - DAILY AGENDA

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

Thursday – December 2

Boehringer Ingelheim Workshop

12:00PM – 10:00PM - Salon ABC Room 5th Floor

Friday – December 3

American College of Veterinary Microbiologists (ACVM)

Examination - 12 PM - 8 PM – Denver/Houston Room - 5th Floor

Boehringer Ingelheim Workshop

6:00 AM - 12 PM - Salon ABC Room 5th Floor

International PRRS Symposium (IPRRSS)

Salons E-F-G-H, 5th Floor - Registration required for attendance.

1:00-2:30 PM - Business meeting Multistate Research Project NC-229

2:00 PM Poster Boards available for poster assembly - Salons F/G/H

2:30-4:00 PM - PRRSV Elimination Workshop/USDA CAP-2 meeting

4:00 PM - Opening Session, Keynote Presentation by Dr. Ralph Baric

5:15PM - 7 PM - IPRRSS Reception/Mixer (cash bar)

5:15 - 7 PM - First Poster Session

Saturday – December 4

American College of Veterinary Microbiologists (ACVM)

Examination - 8 AM - 9 PM - Denver/Houston Room - 5th Floor

Brucellosis Research Group Meeting

7 AM - 8:45 AM - Registration - Salons DA - 5th Floor

8 AM - 5 PM - Salons DA - 5th Floor

NC-1041 Enteric Diseases of Swine and Cattle Meeting

8 AM - 5 PM – Kansas City Room - 5th Floor

International PRRS Symposium (IPRRSS)

Saturday, December 4, Salons E-F-G-H, 5th Floor

Salons E-F-G-H, 5th Floor - Registration required for attendance.

8 AM - 5 PM, Oral Presentations

9:40 AM - Second Poster Session

2:40 PM - Break

Exhibitors - (Table Top) 8 AM – 6 PM - 5th Floor Foyer

Elsevier BV

Eppendorf North America

Qiagen, Inc.

Sarstedt, Inc.

Seppic, Inc.

Tetracore, Inc.

Integrated Special Emphasis Project

Minimizing Antibiotic Resistance Transmission throughout the Food Chain

1:00 PM - 5:00 PM, Northwestern/Ohio Room – 6th Floor

2010 CRWAD AND SATELLITE MEETINGS - DAILY AGENDA

Saturday – December 4 (continued)

CRWAD Sponsorship Committee Meeting

4:00 – 5:30 PM, Minnesota Room – 6th Floor

CRWAD Council Meeting – 5:30 - 9 PM - Great America I Room - 6th Floor

Sunday – December 5

CRWAD Registration – 5th Floor Foyer Registration Booth - 10 AM - 5:30 PM

American Association of Veterinary Immunologists (AAVI)

Board Meeting

8 AM - 12 PM Meeting – Los Angeles Room - 5th Floor

American College of Veterinary Microbiologists (ACVM)

Denver/Houston Room - 5th Floor

8 AM - 10 AM - Examination Committee Meeting

10 AM - 12 PM - Board of Governors Meeting

Brucellosis Research Group Meeting

8:15 AM - 12 PM - Salons DA - 5th Floor

Elsevier Meeting – Editorial Board of Preventive Veterinary Medicine

7:30 AM - 9 AM - Kansas City Room, 5th floor

Integrated Special Emphasis Project

Minimizing Antibiotic Resistance Transmission throughout the Food Chain

8:00 - 11:00 AM, Northwestern/Ohio Room – 6th Floor

NC-1041 Enteric Diseases of Swine and Cattle Meeting

8 AM - 12 PM - Kansas City Room - 5th Floor

PRRSV Diversity (CAP-2 Sub-Project)

8:00 AM to 12:00 PM - Great America I/II Rooms – 6th Floor

AVEPM Schwabe Symposium - A Symposium Honoring the Legacy of Dr. Preben W. Willeberg

Title - Animal health surveillance: from science to application

(Association for Veterinary Epidemiology and Preventive Medicine)

11:30 PM - 5 PM, Chicago Ballroom Salons F/G/H - 5th Floor

12:30 PM, Presentations begin - Open Session

ACVM/AAVI Symposium - Title: Emerging and Re-Emerging Zoonotic Pathogens

1:30 to 5 PM, Chicago Ballroom Salons D/E - 5th Floor - Open Session

CRWAD Researchers Reception and Poster Session I

Reception - Grand Ballroom Salon III - 7th Floor - 6:00 - 8:00 PM

Poster Session I Set-up - 4 PM (Section Posters are listed inside front cover)

Remove posters by 10:00 AM Monday

Poster Session I - 6:30 PM - 8 PM

Exhibitors - (Table Top) 8 AM – 6 PM - 5th Floor Foyer

Monday - December 6

CRWAD Registration - 5th Floor Foyer Registration Table - 7:00 AM - Noon
2:00 PM - 5 PM

CRWAD Sections - 8 AM - 4:30 PM

2010 CRWAD AND SATELLITE MEETINGS - DAILY AGENDA

Monday – December 6 – continued

Exhibits - Break - 4:30 PM - 5 PM – 5th Floor Foyer (review exhibits prior to Poster Session II)

CRWAD Poster Session II - 5:00 PM - 6:30 PM - Grand Ballroom Salon III - 7th Floor

Poster Session II Set-up - 12:00 PM (Section Posters are listed in the Summary Table)

Remove posters immediately upon completion of Poster Session II at 6:30 PM.

AAVI (American Association of Veterinary Immunologists)

Business Meeting and Luncheon - 11:30 AM - 1PM - Buca di Beppo Restaurant

AVEPM Business Meeting - Buffet Luncheon (Members Only)

(Association for Veterinary Epidemiology and Preventive Medicine)

11:30 AM - Great America Room - 6th Floor

Distinguished Veterinary Immunologist Lecture by Dr. Frank Blecha

Kansas State University, Manhattan, KS

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

Exhibitors - (Table Top) 8 AM – 6 PM - 5th Floor Foyer

Tuesday - December 7

Animal Health Research Reviews (AHRR) Board Meeting

7 - 9:30 AM - Great America Room - 6th Floor

CRWAD Registration - 5th Floor Foyer Registration Booth - 8 AM - 11 AM

CRWAD Business Meeting - 11:45 AM - 12:30 PM - Chicago Ballroom A/B/C/D - 5th Floor

Dedication of the Meeting, Introduction of New Members, and Student Competition Awards Presentations.

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

Distinguished Veterinary Microbiologist Lecture to honor Dr. Frederick A. Murphy

University of Texas Medical Branch, Galveston, TX

8:15 AM - Los Angeles/Miami/Scottsdale, 5th Floor

Exhibitors - (Table Top) 8 AM – 10 AM - 5th Floor Foyer

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

2010 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 Friday, Dec. 3 - Tuesday, Dec. 7

POSTER INFORMATION - Poster Sessions I & II - Grand Ballroom III, 7th Floor

SUNDAY POSTER PRESENTERS: December 5, 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, Food and Environmental Safety, and Gastroenteric Diseases Sections will be presented. Please remove your posters by 10:00 AM Monday.

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

Poster boards will be available for poster assembly by noon Monday. Posters for the 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. Poster presenters must furnish their 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

Friday - Saturday - Sunday (afternoon) - Symposiums

Symposiums Schedule:

Friday - Dec. 3, 2:30 PM - 7 PM - International PRRS Symposium Program-Regist. Required

Saturday - Dec. 4, 8 AM - 5:00 PM - Brucellosis Research Program - Regist. Required

Saturday - Dec. 4, 8 AM - 5:00 PM - Int. PRRS Symposium Program - Regist. Required

Sunday - Dec. 5, 1:30 PM - 5 PM - AAVI/ACVM Symposium Program - Open Attendance

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

Sunday - Dec. 5, 8 AM - 12:00 PM - Brucellosis Research Program - Regist. Required

CRWAD Meeting Begins Sunday (evening):

Notice: Section meeting rooms are listed inside front cover

Sunday - Dec. 5 - 6:00-8:00 PM - Kick-Off CRWAD Reception and Poster Session I

Monday - Dec. 6, 8:00 AM - CRWAD Sections Oral Presentations begin in eight separate rooms simultaneously.

Tuesday - Dec. 7, 8:00 AM - CRWAD Sections Oral Presentations begin in eight room.

Tuesday - Dec. 7, 5:00 PM - 6:30 PM - Poster Session II

POSTER PROGRAM

BACTERIAL PATHOGENESIS POSTERS

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

Section Leaders: Jun Lin and 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	Use of Transposon Mutagenesis and Microarray Analysis to identify genes associated with biofilm formation in <i>Actinobacillus pleuropneumoniae</i> .	Y. Tremblay, A. Grasteau, M. Jacques, Université de Montréal, St-Hyacinthe, QC, Canada, <i>Email: mario.jacques@umontreal.ca</i> .
002P	A comparative and functional genomics glimpse of a highly virulent strain of <i>Campylobacter jejuni</i> associated with sheep abortion.	Z. Wu, O. Sahin, Q. Zhang, Department of Veterinary Microbiology and Preventive Medicine, Iowa State University, Ames, IA, <i>Email: wuzw@iastate.edu</i> .
003P	Identification of immunogenic insoluble proteins of <i>Brucella abortus</i> separated by two-dimensional electrophoresis to develop specific antigens required for Brucellosis diagnosis.	K. Ko, J. Kim, J. Kim, H. Lee, J. Park, M. Her, S. Kang, Y. Jang, S. Jung, National Veterinary Research Quarantine Service (NVRQS), Anyang, Korea, Republic of, <i>Email: kimjiyeon75@korea.kr</i> .
004P	Development of the improved multiplex PCR assay for the differential identification of <i>Brucella</i> species	S. Kang, M. Her, J. Kim, J. Kim, K. Ko, B. Ku, Y. Jang, I. Hwang, S. Jung, National Veterinary Research Quarantine Service (NVRQS), Anyang, Korea, Republic of, <i>Email: nvrqsa15@korea.kr</i> .
005P	Gene expression profile of bovine primary mammary epithelial cells infected with <i>Escherichia coli</i> associate with acute or chronic bovine mastitis.	O. Kerro Dego, R. Almeida, D. Luther, S. Oliver, The University of Tennessee, Knoxville, TN, <i>Email: okerrode@utk.edu</i> .
006P	Immune response following vaccination of dairy cows with recombinant <i>Streptococcus uberis</i> adhesion molecule (RSUAM).	R. Almeida, M. Prado, D. Luther, S. Headrick, M. Lewis, S. Oliver, The University of Tennessee, Knoxville, TN; H. Moorehead, The University of Tennessee, Lewisburg, TN, <i>Email: ralmeida@utk.edu</i> .
007P	Cross-reactivity of antibodies against leptospiral recurrent uveitis-associated proteins with ocular proteins.	A. Verma, J. Timoney, B. Stevenson, University of Kentucky, Lexington, KY, <i>Email: averm2@uky.edu</i> .
008P	Immunization with recombinant <i>Brucella abortus</i> outer membrane protein Omp25d, reduces bacterial load after challenge in a murine model for brucellosis.	J. Leonhardt, G. Andrews, University of Wyoming, Laramie, WY; J. Lowry, R. Bowen, Colorado State University, Fort Collins, CO, <i>Email: lowryje@rams.colostate.edu</i> .
009P	<i>Map</i> induces extracellular calcium dependent phagosome acidification to enlist IL-1 β processing and macrophage recruitment.	E. Lamont, S. O'Grady, S. Sreevatsan, University of Minnesota, Saint Paul, MN; T. Eckstein, Colorado State University, Fort Collins, CO, <i>Email: lamo0062@umn.edu</i> .
010P	Enteropathogenic <i>Escherichia coli</i> heat-stable toxin 1 (east1) is not sufficiently virulent to cause diarrhea in neonatal pigs.	X. Ruan, C. Zhang, W. Zhang, South Dakota State University, Brookings, SD; S. Crupper, Emporia State University, Emporia, KS; B. Schultz, D. Robertson, Kansas State University, Manhattan, KS, <i>Email: xsruan@gmail.com</i> .
011P	Protective efficacy of a <i>Francisella tularensis</i> type A complemented O-antigen mutant against murine tularemia.	C. Ryder, G. Berg, T. Inzana, Virginia Tech, Blacksburg, VA, <i>Email: cryder@vt.edu</i> .

(continued)

BACTERIAL PATHOGENESIS POSTERS

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

Section Leaders: Jun Lin and Gireesh Rajashekara

No.	Title	Authors
012P	The <i>invA</i> gene (BMEI0215) of <i>Brucella melitensis</i> is needed for intracellular replication, but not for invasion.	J. Alva-Pérez, B. Arellano-Reynoso, F. Suárez Guemes, Universidad Nacional Autonoma de Mexico, Mexico DF., Mexico; R. Hernández-Castro, Hospital General Dr. Manuel Gea Gonzalez, Mexico DF., Mexico; J. Alva-Pérez, B. Arellano-Reynoso, F. Suárez Guemes, Universidad Nacional Autonoma de Mexico, Mexico DF., Mexico, <i>Email: jorgealvap1712@hotmail.com</i> .
013P	<i>Citrobacter rodentium</i> causes structural and functional alterations in conditionally immortalized Ptk6 colonic epithelial cells.	E. Gart, B. Schultz, L. Willard, S. Narayanan, Kansas State University, Manhattan, KS, <i>Email: egart@ksu.edu</i> .
014P	Application of change-mediated antigen technology (CMAT) in the identification of <i>Francisella tularensis</i> gene products up-regulated during infection.	G. Vernati, G. Andrews, University of Wyoming, Laramie, WY, <i>Email: gandrews@uwyo.edu</i> .
015P	Microbial protein-Antigenome Determination (MAD) technology: A proteomics-based strategy for rapid identification of microbial targets of host humoral immune responses.	I. Kudva, National Animal Disease Center, USDA, Ames, IA; B. Krastins, D. Sarracino, Thermo Fisher Scientific, Cambridge, MA; R. Griffin, S. Calderwood, Massachusetts General Hospital, Boston, MA; H. Sheng, C. Hovde, University of Idaho, Moscow, ID; M. John, Pathovacs, Inc., Ames, IA, <i>Email: Indira.Kudva@ars.usda.gov</i> .
016P	Detecting differential protein expression between pathogenic and commensal <i>Staphylococcus aureus</i> using SILAC.	M. Manickam, I. Mullarky, Virginia Polytechnic Institute and State University, Blacksburg, VA, <i>Email: mmanisha@vt.edu</i> .
017P	On the Trail of Regulatory T cells in Blood and Tissues of <i>Mycobacterium paratuberculosis</i> Infected Cattle.	B. Murphy, J. Roussey, N. Turk, S. Sipkovsky, C. Colvin, P. Coussens, Michigan State University, East Lansing, MI, <i>Email: murph398@msu.edu</i> .
018P	Highly pathogenic strains of <i>Salmonella</i> Enteritidis show enhanced tolerance to acid, oxidative stress and better survival in egg albumen.	D. Shah, Department of Veterinary Microbiology and Pathology, College of Veterinary Medicine, Washington State University, Pullman, WA; Q. Hawley, College of Veterinary Medicine, North Carolina State University, Raleigh, NC; T. Addwebi, C. Casavant, Washington State University, College of Veterinary Medicine, Pullman, WA, <i>Email: dshah@vetmed.wsu.edu</i> .
019P	Evaluation of three real-time PCR assays for detection of pathogenic <i>Leptospira</i> species in canine urine samples	J. Fink, R. Vemulapalli, R. Landau, G. Moore, Purdue University, West Lafayette, IN; C. Santrich, Purdue University, Animal Disease Diagnostic Laboratory, West Lafayette, IN, <i>Email: fink1@purdue.edu</i> .
020P	Development of Cpb2 diagnostic ELISA and evaluation of the role of Cpb2 toxin in enteric disease in neonatal piglets.	J. Kircanski, D. Hodgins, Y. Pei, V. Parreira, J. Prescott, University of Guelph, Guelph, ON, Canada, <i>Email: jkircans@uoguelph.ca</i> .

(continued)

BACTERIAL PATHOGENESIS POSTERS

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

Section Leaders: Jun Lin and Gireesh Rajashekara

No.	Title	Authors
021P	Sub-inhibitory concentrations of the antibiotic florfenicol reduces invasion in isolates of multi-drug resistant <i>Salmonella</i> Typhimurium DT104.	B. Brunelle, S. Bearson, National Animal Disease Center, Ames, IA; B. Bearson, National Laboratory for Agriculture and the Environment, Ames, IA, <i>Email: brian.brunelle@ars.usda.gov</i> .
022P	Peptide nucleic acids inhibit <i>Brucella suis</i> in pure culture and infected macrophages.	M. Seleem, Institute for Critical Technology and Applied Science, Blacksburg, VA; N. Jain, J. Alexander, N. Sriranganathan, S. Boyle, Virginia-Maryland Regional College of Veterinary Medicine, Blacksburg, VA; R. Wattam, J. Setubal, Virginia Bioinformatics Institute, Blacksburg, VA, <i>Email: icaalex01@vt.edu</i> .
023P	Diversity of <i>Enterococcus cecorum</i> in chickens from Ontario	V. Nicholson, P. Boerlin, University of Guelph, Ontario Veterinary College, Guelph, ON, Canada; D. Slavic, M. Brash, University of Guelph, Animal Health Laboratory, Guelph, ON, Canada; B. Sanei, Ontario Ministry of Agriculture, Food and Rural Affairs, Guelph, ON, Canada, <i>Email: vivianni@uoquelp.ca</i> .
024P	Problems of Epidemiological studies on Q fever in the Odessa region of Ukraine.	E. Volosyanko, L. Marushchak, L. Dedok, State Research Institute of Laboratory Diagnostics and Veterinary Expertise, Kiev, Ukraine; Z. Kushnir, N. Bek, Lvov Research Institute of Epidemiology and Hygiene, Lvov, Ukraine; A. Volkov, V. Ringach, Odessa Oblast Sanitary and Epidemiological Station, Odessa, Ukraine, <i>Email: Maruschak@vandex.ru</i> .

BIOSAFETY AND BIOSECURITY POSTERS

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

Section Leaders: Scott Dee and 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 is required.

No.	Title	Authors
025P	Application of alternative methods of body temperature measurement in swine.	M. Allerson, University of Minnesota, St. Paul, MN, <i>Email: alle0482@umn.edu</i> .
026P	Animal genetics rescue from diseased animals using somatic cell nuclear transfer (SCNT) technology	K. Gregg, T. Xiang, S. Arenivas, E. Hwang, F. Arenivas, A. Picou, S. Walker, I. Polejaeva, Viagen, Austin, TX, <i>Email: keqin.gregg@viagen.com</i> .

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
027P	Associations of antimicrobial use and antimicrobial resistance in <i>Escherichia Coli</i> isolates individually sampled from feedlot cattle.	K. Benedict, P. Morley, Colorado State University, Fort Collins, CO; S. Gow, Public Health Agency of Canada, Saskatoon, SK, Canada; C. Booker, Feedlot Health Management Services, Okotoks, AB, Canada; T. McAllister, University of Lethbridge, Lethbridge, AB, Canada, <i>Email: kbened@colostate.edu</i> .
028P	Isolation and characterization of methicillin resistant <i>Staphylococcus aureus</i> from bulk tank milk in Minnesota dairy farms.	P. Haran, S. Godden, J. Bender, S. Sreevatsan, University of Minnesota, St .Paul, MN, <i>Email: hara0110@umn.edu</i> .
029P	Molecular characteristics of Ukrainian Laboratory and industrial strains of <i>Bacillus Anthracis</i> .	V. Skrypyk, O. Deriabin, O. Deryabina, A. Skrypyk, N. Parkhomenko, Institute for Veterinary Medicine, Kyiv, Ukraine, <i>Email: skrip2002@inbox.ru</i> .
030P	Direct and indirect contact rates among Vermont dairy farms.	J. Smith, University of Vermont, Burlington, VT, <i>Email: julie.m.smith@uvm.edu</i> .
031P	Vaccination of calves up to 15 months of age against <i>Mycobacterium avium ssp. paratuberculosis</i> impacts Johne's disease incidence and cull rates.	K. Esch, R. Royer, M. Kuenne, Elkader Veterinary Clinic, Elkader, IA; J. Schiltz, C. Thoen, Iowa State University, Ames, IA, <i>Email: cthoen@iastate.edu</i> .
032P	First report of the bovine lymphotropic herpesvirus (BLHV) within Canada and detection of the viral genome in tissues of a bovine aborted fetus.	N. Music, O. Allam, R. Drolet, D. Tremblay, C. Gagnon, Faculté de médecine vétérinaire, Université de Montréal, Saint-Hyacinthe, QC, Canada, <i>Email: nedzad.music@umontreal.ca</i> .
033P	Factors associated with bovine liver condemnations in Ontario Provincial Abattoirs 2001-2007.	G. Alton, D. Pearl, K. Bateman, O. Berke, University of Guelph, Guelph, ON, Canada; W. McNab, Ontario Ministry of Agriculture, Food and Rural Affairs, Guelph, ON, Canada, <i>Email: altong@uoguelph.ca</i> .
034P	Multiple introductions of North American type 2 porcine reproductive and respiratory syndrome viruses into Thailand	H. Tun, C. Wong, M. Shi, F. Leung, School of Biological Sciences, The University of Hong Kong, Hong Kong, Hong Kong; A. Amonsin, Emerging and Re-emerging Diseases in Animals, Research Unit, Faculty of Veterinary Science, Chulalongkorn University, Bangkok, Thailand, <i>Email: harry.hku@hku.hk</i> .
035P	A web-based database and phylogenetic tools to study molecular epidemiology and evolution of Porcine Reproductive and Respiratory Syndrome Virus (PRRSV).	C. Wong, J. Li, T. Lam, M. Shi, F. Leung, The University of Hong Kong, Hong Kong, Hong Kong, <i>Email: cyin3672@hku.hk</i> .
036P	Case study of two vector-borne diseases in humans and animals of the far North region of Cameroon: Implications for preventative measures.	E. Walz, R. Garabed, College of Veterinary Medicine, The Ohio State University, Columbus, OH; D. Ewing, M. Moritz, Department of Anthropology, The Ohio State University, Columbus, OH; W. Alhaji Lawan, Centre d'Appui à la Recherche et au Pastoralisme, Maroua, Cameroon, <i>Email: walz.emily@gmail.com</i> .

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
037P	Situation of Rabies in Thailand, 2009.	A. Banjong, W. Kachen, P. Tippawon, W. Vilaiporn, FETPV Department of Livestock Development, Bangkok, Thailand; C. Karoon, Veterinary Epidemiology Development center, Department of Livestock Devepment, Bangkok, Thailand, <i>Email: vet0180@hotmail.com</i> .
038P	A mathematical model to predict the effect of antimicrobials on the efficiency of horizontal gene transfer.	G. Peterson, R. Gehring, M. Lawrence, J. Coetzee, S. Narayanan, Kansas State University, Manhattan, KS, <i>Email: gpeterso@vet.ksu.edu</i> .
039P	Monitoring of Viral Haemorrhagic Septicaemia of Rainbow Trout in Ukraine.	O. Deryabin, O. Gaidei, Institute for Veterinary Medicine, Kyiv, Ukraine; A. Golovko, National Academy of Agrarian Sciences of Ukraine, Kyiv, Ukraine, <i>Email: don.lmb@gmail.com</i> .
040P	Nasal shedding of Equine Herpesvirus-1 from horses in an outbreak of Equine Herpes Myeloencephalopathy in Western Canada.	B. Burgess, D. Lunn, S. Hussey, P. Morley, Colorado State University, Fort Collins, CO; N. Tokateloff, K. Poirier, S. Manning, K. Lohmann, University of Saskatchewan, Saskatoon, SK, Canada, <i>Email: brandy.burgess@colostate.edu</i> .
041P	Cluster analysis of methicillin resistant Staphylococci isolated from dogs and cats in Iowa.	T. Frana, N. Boyes, J. Garza, J. Kinyon, Iowa State University, Ames, IA, <i>Email: tfrana@iastate.edu</i> .

FOOD AND ENVIRONMENTAL SAFETY POSTERS

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

Section Leader: Yvette Johnson

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
042P	Bulk tank bacteria and selected foodborne pathogens in raw milk of Wisconsin farmstead dairy producers.	A. Rodrigues, J. Pantoja, C. Hulland, P. Ruegg, University of Wisconsin, Madison, WI, <i>Email: acorvet@yahoo.com</i> .
043P	Factors associated with coliform count in unpasteurized milk	J. Pantoja, P. Ruegg, D. Reinemann, University of Wisconsin-Madison, Madison, WI, <i>Email: jpantoja@wisc.edu</i> .
044P	Methicillin resistant Staphylococci found in clinical mastitis samples from dairy cattle in Iowa.	A. Beahm, J. Kinyon, T. Frana, Iowa State University, Ames, IA, <i>Email: tfrana@iastate.edu</i> .
045P	Acute host stress increases horizontally mobilizable plasmids and antimicrobial resistance genes in cattle feces.	S. Menon, R. Mosher, C. Cull, G. Peterson, A. Kumar, J. Coetzee, S. Narayanan, Kansas State University, Manhattan, KS, <i>Email: smenon@vet.k-state.edu</i> .
046P	Transcriptional response of <i>Campylobacter jejuni</i> to erythromycin exposure.	Q. Xia, Z. Shen, Z. Wu, W. Muraoka, R. Sippy, Q. Zhang, Iowa State University, Ames, IA, <i>Email: qxia@iastate.edu</i> .
047P	Comparative studies of heavy metal concentration in Prawn and Water samples from Epe Lagoon and Asejire Rivers in Nigeria.	O. Adedeji, R. Okocha., University of Ibadan, Nigeria, Ibadan, Nigeria, <i>Email: oluadedeji2001@yahoo.com</i> .
048P	Antimicrobial resistance and resistance genes in <i>E. coli</i> and <i>Salmonella</i> isolated from salmon and shrimp purchased in Canada.	N. Janecko, R. Reid-Smith, A. Desruisseau, B. Avery, Laboratory of Foodborne Zoonoses, Public Health Agency of Canada, Guelph, ON, Canada; F. Uhland, Faculte de Medicine Veterinaire, Universite du Montreal, Montreal, QC, Canada; P. Boerlin, Dept. of Pathobiology, University of Guelph, Guelph, ON, Canada; S. McEwen, Dept. of Population Medicine, University of Guelph, Guelph, ON, Canada, <i>Email: nicol.janecko@phac-aspc.gc.ca</i> .
049P	Presence of <i>Listeria Monocytogenes</i> and <i>Salmonella</i> in ready to eat meats from processing plants and retail outlets in North Dakota.	A. Ntaate, D. Doetkott, M. Khaita, North Dakota State University, Fargo, ND, <i>Email: Anthony.Ntaate@ndsu.edu</i> .
050P	Characterization and distribution of <i>Salmonella</i> from egg layer and pullet grower operations in Ontario, Canada, by MLVA molecular typing.	C. Leon-Velarde, S. Chen, Laboratory Services Division, University of Guelph, Guelph, ON, Canada; F. Olea-Popelka, Department of Clinical Sciences, College of Veterinary Medicine and Biomedical Sciences, Animal Population Health Institute, Colorado State University, Fort Collins, CO; J. Odumeru, Laboratory Services Branch, Ministry of the Environment, Etobicoke, ON, Canada, <i>Email: cleonvel@uoguelph.ca</i> .
051P	Comparison of RT PCR with three culture methods for detection of <i>Salmonella</i> from poultry environmental samples.	D. Adams, C. Thompson, L. McDeid, M. Savala, E. Strait, T. Frana, Iowa State University, Ames, IA, <i>Email: tfrana@iastate.edu</i> .

GASTROENTERIC DISEASES POSTERS

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

Section Leaders: David H. Francis and Radhey S. Kaushik

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
053P	Protective effects of a transgenic carrot vaccine on piglet diarrhea.	J. Han, Y. Kim, Kangwon National University, Chuncheon, Korea, Republic of, <i>Email: hanjh@kangwon.ac.kr</i> .
054P	Application of a 'FaeG-FedF-LT _B ' adhesin-toxin fusion antigen in vaccine development against enterotoxigenic <i>Escherichia coli</i> associated diarrhea in pigs.	X. Ruan, C. Zhang, W. Zhang, South Dakota State University, Brookings, SD; T. Casey, National Animal Disease Center, Ames, IA, <i>Email: xruan@jacks.sdstate.edu</i> .
055P	Genotypic comparison of virulent extraintestinal pathogenic <i>Escherichia coli</i> isolates causing fetal pneumonia in animals with those from healthy human.	C. DebRoy, E. Roberts, S. Kariyawasam, Pennsylvania State University, University Park, PA; E. de Muinck, University of Oslo, Oslo, Norway, <i>Email: rcd3@psu.edu</i> .
056P	Morphological and Structural Changes in Colon of adult FVB Mice Infected with <i>Citrobacter rodentium</i> .	E. Gart, B. Schultz, L. Willard, S. Narayanan, Kansas State University, Manhattan, KS, <i>Email: egart@ksu.edu</i> .
057P	Identification of <i>Helicobacter suis</i> in pig-producing regions of North America.	L. Kopta, J. Paquette, T. Bowersock, L. Choromanski, J. Galvin, D. Foss, Pfizer Animal Health, Kalamazoo, MI, <i>Email: dennis.l.foss@pfizer.com</i> .
058P	Experimental infection of swine with <i>Helicobacter suis</i> .	T. Bowersock, D. Peterson, M. Sanchez, L. Kopta, J. Paquette, L. Choromanski, R. Laurinat, J. Galvin, L. Taylor, D. Foss, Pfizer Animal Health, Kalamazoo, MI, <i>Email: dennis.l.foss@pfizer.com</i> .
059P	Deletion of glucose-inhibited division gene (gidA) alters the morphological and replication characteristics of <i>Salmonella enterica</i> serovar Typhimurium.	D. Shippy, N. Eakley, J. Heintz, R. Albrecht, A. Fadl, University of Wisconsin-Madison, Madison, WI, <i>Email: dshippy@wisc.edu</i> .
060P	Comparison of selenite and Rappaport-Vassiliadis enrichment methods for isolation of <i>Salmonella</i> from dairy cattle naturally infected with <i>Salmonella</i> Newport MDR-AmpC.	D. Short, M. Kristula, D. Galligan, S. Young, S. Rankin, H. Aceto, University of Pennsylvania School of Veterinary Medicine, Kennett Square, PA, <i>Email: helenwa@vet.upenn.edu</i> .
061P	Phylogenetic analysis of the 3D region of porcine kobuviruses detected from Korean diarrheic pigs.	S. Park, H. Kim, S. Rho, J. Han, V. Nguyen, B. Park, Seoul National University, Seoul, Korea, Republic of, <i>Email: juni1212@snu.ac.kr</i> .
062P	Identification of the receptor-binding domain (RBD) of the porcine epidemic diarrhea virus spike protein	D. Lee, C. Lee, Kyungpook National University, Daegu, Korea, Republic of, <i>Email: gulbay138@knu.ac.kr</i> .

IMMUNOLOGY POSTERS

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

Section Leader: Isis Mullarky

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
063P	The US Veterinary Immune Reagent Network: Update on reagents for the horse.	B. Wagner, S. Babasyan, J. Hillegas, E. Kabithe, Cornell University, Ithaca, NY; J. LaBresh, Kingfisher Biotech, St. Paul, MN; D. Tompkins, C. Baldwin, University of Massachusetts, Amherst, MA, <i>Email: bw73@cornell.edu</i> .
064P	Swine toolkit progress for the US Veterinary Immune Reagent Network.	J. Lunney, P. Boyd, A. Crossman, USDA ARS BARC APDL, Beltsville, MD; J. LaBresh, Y. Sullivan, Kingfisher Biotech Inc, St. Paul, MN; L. Kakach, Kingfisher Biotech Inc., St. Paul, MN; B. Wagner, Cornell University, Ithaca, NY; H. Dawson, USDA ARS BARC BHNRC DGIL, Beltsville, MD; D. Tompkins, T. Hudgens, C. Baldwin, University of Massachusetts, Amherst, MA, <i>Email: Joan.Lunney@ars.usda.gov</i> .
065P	Comparison of agreement rate between developed C-ELISA and commercial C-ELISA assay for serological diagnosis of bovine Brucellosis.	J. Kim, K. Ko, D. Cho, M. Her, J. Kim, S. Kang, B. Ku, I. Hwang, Y. Jang, S. Jung, National Veterinary Research Quarantine Service (NVRQS), Anyang, Korea, Republic of, <i>Email: kimjiyeon75@korea.kr</i> .
066P	Development of specific antigens for serodiagnosis of <i>Mycobacterium bovis</i> using latex bead agglutination assay.	Y. Jang, B. Ku, P. Kim, M. Her, J. Kim, J. Kim, I. Hwang, S. Kang, K. Ko, S. Jung, Y. Cho, National Veterinary Research Quarantine Service (NVRQS), Anyang, Korea, Republic of, <i>Email: kimiiveon75@korea.kr</i> .
067P	Development of a skin test to map equine infectious anemia virus (EIAV) envelope-specific immune responses <i>in vivo</i> .	C. Liu, S. Cook, A. Adams, D. Horohov, University of Kentucky, Lexington, KY, <i>Email: cliuc@uky.edu</i> .
068P	Do the criteria used to interpret the microscopic agglutination test (MAT) for the diagnosis of canine Leptospirosis need to be changed?	E. Mukhtar, L. Larson, O. Okwumabua, R. Schultz, University of Wisconsin-Madison, Madison, WI, <i>Email: LarsonL@svm.vetmed.wisc.edu</i> .
069P	Mycobacterial immunodominant antigens ESAT6 and CFP10 improve tuberculin skin test specificity in cattle naturally infected.	S. Flores Villalva, F. Suárez Güemes, J. Gutierrez Pabello, Laboratorio de Investigación en Tuberculosis y Brucelosis, Facultad de Medicina Veterinaria y Zootecnia, UNAM, D.F., Mexico; C. Espitia, Instituto de Investigaciones Biomédicas, UNAM, D.F., Mexico; M. Vordermeier, TB Research Group, Veterinary Laboratories Agency, Weybridge, Addlestone, Surrey, United Kingdom, <i>Email: fv.susana@gmail.com</i> .
070P	The probiotic strain <i>Lactobacillus rhamnosus</i> GG has anti-viral effects against three canine viruses	E. Ephraim, L. Larson, R. Schultz, University of Wisconsin, Madison, WI, <i>Email: edenephraim2002@yahoo.com</i> .

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IMMUNOLOGY POSTERS

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

Section Leader: Isis Mullarky

No.	Title	Authors
071P	Serological survey of the southern Wisconsin raccoon population to <i>Leptospira</i> , canine parvovirus type 2, and canine distemper Virus.	B. Thiel, L. Larson, R. Schultz, Dept. of Pathobiological Sciences, School of Veterinary Medicine, University of Wisconsin-Madison, Madison, WI; O. Okwumabua, Wisconsin Veterinary Diagnostic Laboratory, Madison, WI, <i>Email: bethiel@wisc.edu</i> .
072P	Antibody and interferon- γ responses of pigs vaccinated with killed, adjuvanted H3N2 Swine Influenza Virus (SIV) vaccine.	D. Foss, R. Yancey, Jr., D. Mwangi, J. Czach, T. Hildebrand, M. Kievit, M. Aaron, M. Wyant, C. Lenz, D. Slade, J. Bubolz, T. Bowersock, L. Taylor, V. Rapp-Gabrielson, Pfizer Animal Health, Kalamazoo, MI, <i>Email: dennis.l.foss@pfizer.com</i> .
073P	Immunologic responses to <i>Mycobacterium avium</i> subsp. <i>paratuberculosis</i> protein cocktail vaccines in a mouse model.	A. Barnhill, J. Bannantine, D. Bayles, J. Stabel, NADC, Ames, IA; Y. Chang, Cornell University, Ithaca, NY; M. Osman, Iowa State University, Ames, IA, <i>Email: alison.barnhill@ars.usda.gov</i> .
074P	Infection of B cells by <i>Brucella abortus</i> .	M. Ramírez-Saldaña, M. Moreno-Lafont, M. Aguilar-Santelises, R. López-Santiago, Escuela Nacional de Ciencias Biológicas - IPN, México DF, Mexico, <i>Email: rlslenon@gmail.com</i> .
075P	Monocytes: The precursors of inflammatory dendritic cells in <i>Staphylococcus aureus</i> infection.	M. Bharathan, N. Sriranganathan, I. Mullarky, Virginia Polytechnic Institute and State University, Blacksburg, VA; W. Mwangi, Texas A&M University, College Station, TX, <i>Email: mini@vt.edu</i> .
076P	Characterization of <i>Brucella abortus</i> infection of bovine monocyte-derived dendritic cells.	M. Heller, J. Watson, M. Blanchard, K. Jackson, J. Stott, R. Tsois, University of California Davis, Davis, CA, <i>Email: mcheller@ucdavis.edu</i> .
077P	Proteomic analysis of Bovine Viral Diarrhea Virus infected monocytes.	L. Pinchuk, M. Ammari, F. McCarthy, B. Nanduri, Mississippi State University, Starkville, MS, <i>Email: pinchuk@cvm.msstate.edu</i> .
078P	Ethyl pyruvate diminishes the endotoxin-induced inflammatory response of bovine mammary endothelial cells.	C. Corl, H. Robinson, G. Contreras, S. Holcombe, V. Cook, L. Sordillo, Michigan State University, East Lansing, MI, <i>Email: corl@msu.edu</i> .
079P	Selenoenzyme status affects eicosanoid biosynthesis in macrophages	S. Mattmiller, C. Corl, L. Sordillo, Michigan State University, East Lansing, MI; B. Carlson, National Cancer Institute, Bethesda, MD, <i>Email: mattmill@msu.edu</i> .
080P	Activation of porcine small intestinal epithelial IPEC-1 cells with bacteria-associated toll-like receptor ligands.	C. Sreenivasan, R. Kaushik, South Dakota State University, Brookings, SD, <i>Email: radhey.kaushik@sdsu.edu</i> .
081P	Comparative proteomic analysis of protein modulation in bovine lung tissue during experimentally induced Mannheimia haemolytica pneumonia.	E. Tall, J. Ward, J. Boehmer, U.S. Food and Drug Administration Center for Veterinary Medicine, Laurel, MD, <i>Email: elizabeth.tall@fda.hhs.gov</i> .
082P	Equine hematopoietic progenitor stem cells are enriched in lineage-negative populations of cord blood mononuclear cells.	J. Watson, K. Jackson, D. Borjesson, UC Davis, Davis, CA, <i>Email: jlwatson@ucdavis.edu</i> .

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IMMUNOLOGY POSTERS

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

Section Leader: Isis Mullarky

No.	Title	Authors
083P	Characterization of equine humoral antibody response to the nonstructural proteins of equine arteritis virus	Y. Go, P. Timoney, U. Balasuriya, University of Kentucky, Lexington, KY; E. Snijder, Leiden University Medical Center, Leiden, Netherlands, <i>Email: go.yun@uky.edu</i> .
084P	Humoral immune response to <i>Saprolegnia parasitica</i> in rainbow trout (<i>Oncorhynchus mykiss</i>).	V. García-Flores, M. Vega-Ramírez, M. Moreno-Lafont, R. López-Santiago, Escuela Nacional de Ciencias Biológicas - IPN, México DF, Mexico; J. Damas-Aguilar, E. Rivas-González, El Zarco - SAGARPA, Toluca, Mexico, <i>Email: rslennon@gmail.com</i> .
085P	Comparison of humoral and cellular immune responses to inactivated swine influenza virus vaccine in weaned pigs.	R. Platt, P. Gauger, K. Kimura, J. Roth, Iowa State University, Ames, IA; A. Vincent, C. Loving, E. Zanella, K. Lager, M. Kehrl, National Animal Disease Center, Ames, IA, <i>Email: rplatt@iastate.edu</i> .
086P	Pro-inflammatory and pro-apoptotic responses of TNF- α stimulated bovine mammary endothelial cells	S. Aitken, C. Corl, L. Sordillo, Michigan State University, East Lansing, MI, <i>Email: aitkenst@cvm.msu.edu</i> .
087P	Immune and genetic control of swine responses to Porcine Reproductive and Respiratory Syndrome Virus Infection.	J. Lunney, H. Chen, USDA ARS BARC APDL, Beltsville, MD; J. Steibel, Michigan State Univ., East Lansing, MI; J. Reecy, E. Fritz, M. Rothschild, Iowa State Univ., Ames, IA; M. Kerrigan, B. Tribble, R. Rowland, Kansas State Univ., Manhattan, KS, <i>Email: Joan.Lunney@ars.usda.gov</i> .
088P	Antibody responses to BVDV in persistently infected (PI) cattle vaccinated with a combination of commercial and experimental BVDV vaccines.	D. Sudbrink, B. Thiel, S. Schultz, L. Larson, K. Kurth, C. Haase, R. Schultz, University of Wisconsin-Madison, Madison, WI, <i>Email: LarsonL@svm.vetmed.wisc.edu</i> .
089P	A comparative field study of BVDV Type 1 and 2, and BHV-1 immunologic memory to infectious (MLV) and non-infectious (killed) vaccines in dairy cows.	C. Haase, R. Schultz, University of Wisconsin-Madison, Madison, WI, <i>Email: rschultz@svm.vetmed.wisc.edu</i> .
090P	Comparison of the efficacy of single or booster vaccination of Bison with <i>Brucella Abortus</i> Strain RB51.	S. Olsen, National Animal Disease Center, Ames, IA, <i>Email: Steven.olsen@ars.usda.gov</i> .
091P	Comparison of immune responses in aged horses given commercially available live or inactivated equine influenza (EI) vaccines.	A. Adams, S. Reedy, D. Horohov, The Gluck Equine Research Center, Lexington, KY; M. Lean, Aberystwyth University, Institute of Biological, Environmental & Rural Sciences, Aberystwyth, United Kingdom, <i>Email: amanda.adams@uky.edu</i> .
092P	Absence of glycan moieties in GP3 & GP5 of a PRRSV field isolate enhances its susceptibility to antibody neutralization and its ability to elicit neutralizing antibody response	H. Vu, B. Kwon, A. Pattnaik, F. Osorio, University of Nebraska-Lincoln, Lincoln, NE; K. Yoon, Iowa State University, Ames, IA; W. Laegreid, University of Illinois at Urbana-Champaign, Urbana, IL, <i>Email: hiepvu07@gmail.com</i> .

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IMMUNOLOGY POSTERS

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

Section Leader: Isis Mullarky

No.	Title	Authors
093P	Potential T-cell epitopes present in nonstructural proteins 9 and 10 of type-II Porcine Reproductive and Respiratory Syndrome Virus eliciting IFN- γ response.	R. Parida, D. Peterson, A. Pattnaik, F. Osorio, University of Nebraska-Lincoln, Lincoln, NE; I. Choi, Konkuk University, Seoul, Korea, Republic of, <i>Email: rparida@unlnotes.unl.edu</i> .
094P	Use of attenuated <i>Erysipelothrix rhusiopathiae</i> strains as vectors for <i>in vivo</i> -delivery of porcine IL-18 for immunomodulation.	Y. Shimoji, Y. Ogawa, Y. Muneta, National Institute of Animal Health, Tsukuba, Ibaraki, Japan, <i>Email: shimoji@affrc.go.jp</i> .
095P	IL-1 β cooperates with TGF- β to induce invasion and stem cell formation in Gliomas.	j. shi, X. Wang, L. Wang, Kansas State University, Manhattan, KS, <i>Email: jshi@vet.k-state.edu</i> .
096P	IL-1 β cooperates with TGF- β to induce invasion and stem cell formation in Gliomas.	L. Wang, Z. Liu, G. Seo, M. Pyle, D. Troyer, J. Shi, Kansas State University, Manhattan, KS, <i>Email: jshi@vet.k-state.edu</i> .

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
097P	Bactericidal effect of tracheal antimicrobial peptide against bovine respiratory pathogens.	K. Taha-Abdelaziz, D. Slavic, J. Caswell, University of Guelph, Guelph, ON, Canada; J. Perez-Casal, Vaccine and Infectious Disease Organization, Saskatoon, SK, Canada, <i>Email: kabdelaz@uoguelph.ca</i> .
098P	Isolation of <i>Histophilus somni</i> from the nasal exudate of a clinically healthy adult goat.	N. Pérez-Romero, E. Díaz-Aparicio, F. Aguilar-Romero, CENID Microbiología, INIFAP-SAGARPA, Mexico, D.F, Mexico; R. Hernández-Castro, Dirección de Investigación, Hospital General Dr. Manuel Gea González, Secretaría de Salud, Mexico, D.F, Mexico; B. Arellano-Reynoso, Universidad Nacional Autónoma de México, Mexico, D.F, Mexico; R. Morales-Cortés, Comité de fomento y protección Pecuaria del Estado de Puebla, Puebla, Mexico, <i>Email: arerev@yahoo.com</i> .
099P	The role of the white-tailed deer immune response in the presence of BVDV infection.	J. Mediger, C. Chase, South Dakota State University, Brookings, SD; K. Fulk, P. Federico, B. Pesch, J. Ridpath, National Animal Disease Lab, USDA, Ames, IA, <i>Email: jmediger@hotmail.com</i> .
100P	Antigenic variations associated with poor performance of direct fluorescent antibody test to detect bovine viral diarrhea virus (BVDV) II antigen in fresh tissues.	L. Yan, B. Baughman, L. Pace, M. Zhang, Mississippi State University, Pearl, MS; S. Zhang, Texas A&M University, College Station, TX, <i>Email: ShupingZhang@cvm.tamu.edu</i> .
101P	Adenosine-5'-triphosphate release by lipopolysaccharide and interleukin-1 stimulated bovine lung epithelial cells	D. McClenahan, M. Craddick, J. Dubbert, A. Lower, University of Northern Iowa, Cedar Falls, IA; M. Ackermann, Iowa State University, Ames, IA, <i>Email: david.mcclenahan@uni.edu</i> .
102P	Generation of human telomerase reverse transcriptase-immortalized porcine monocyte/macrophage cell lines.	M. Sagong, c. Lee, Kyungpook National University, Daegu, Korea, Republic of, <i>Email: fiog40@naver.com</i> .
103P	Preliminary study of PRRSV inactivated vaccine efficacy in vaccinated piglets.	M. Yeom, H. Kim, B. Park, Seoul National University, Seoul, Korea, Republic of; H. Moon, S. Han, D. Son, J. Hwang, T. Oh, Y. Lee, J. Kim, B. Kang, D. Song, Green Cross Veterinary Products, Yongin, Korea, Republic of, <i>Email: paransearo@naver.com</i> .
104P	Genetic diversity of Porcine Reproductive and Respiratory Syndrome Virus in Korea.	E. CHOI, C. LEE, J. SONG, S. Cha, National Veterinary Research and Quarantine Service, Anyang, Korea, Republic of; H. SONG, Chonbuk National University, Jeonju, Korea, Republic of, <i>Email: choiej@korea.kr</i> .

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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
105P	Detection of Porcine Reproductive and Respiratory Syndrome Virus using a DNA microarray chip.	E. CHOI, C. LEE, J. SONG, National Veterinary Research and Quarantine Service, Anyang, Korea, Republic of; J. Kim, H. Joo, JenoBiotech Incorporation, Chuncheon, Korea, Republic of; T. Kim, Biometrix Technology, Chuncheon, Korea, Republic of; H. SONG, Chonbuk National University, Jeonju, Korea, Republic of, <i>Email: choiej@korea.kr</i> .
106P	Sensitivity and specificity of ELISA tests for serological diagnosis of PRRSV Type 1.	T. Stadejek, E. Skrzypiec, K. Chabros, K. Podgorska, Z. Pejsak, National Veterinary Research Institute, Pulawy, Poland, <i>Email: stadejek@piwet.pulawy.pl</i> .
107P	Serological efficacy of Circumvent™ PCV in pigs from Korea.	Y. Kim, J. Han, Kangwon National University, Chuncheon, Korea, Republic of, <i>Email: kslippy@daum.net</i> .
108P	Results of Cicumvent™ PCV in pigs on growth performance and mortality rate.	Y. Kim, J. Han, Kangwon National University, Chuncheon, Korea, Republic of; M. Kim, Intervet Schering-Plough Korea, Seoul, Korea, Republic of, <i>Email: kslippy@daum.net</i> .
109P	Prevalence of antibodies to avian influenza viruses and risk factors for exposure in Thai free-grazing duck flocks.	A. Beaudoin, R. Singer, J. Bender, University of Minnesota College of Veterinary Medicine, Saint Paul, MN; J. Sasipreeyajan, P. Kitikoon, Chulalongkorn University Faculty of Veterinary Sciences, Bangkok, Thailand; S. Pakinsee, Chulalongkorn University College of Public Health, Bangkok, Thailand, <i>Email: beau0209@umn.edu</i> .

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
110P	Serological and molecular detection of equine piroplasms in Korea	D. Kwak, Kyungpook National University College of Veterinary Medicine, Daegu, Korea, Republic of, <i>Email: dmkwak@knu.ac.kr</i> .
111P	The effect of genetics of <i>Mycoplasma haemolamae</i> on virulence, transmission, and detection.	R. Pentecost, A. Marsh, J. Daniels, P. Rajala-Schultz, J. Lakritz, Ohio State University, Columbus, OH, <i>Email: pentecost.3@osu.edu</i> .

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
112P	System for viral and mycoplasma contamination control of veterinary preparations and row materials.	R. Dotsenko, A. Gerilovych, A. Stegnyy, V. Bolotin, O. Solodyankin, S. Vovk, NSC Institute of experimental and clinical veterinary medicine, Kharkiv, Ukraine, <i>Email: antger@rambler.ru</i> .
113P	RNA interference of feline herpesvirus by synthetic siRNAs in corneal epithelial cells.	R. Wilkes, The University of Tennessee College of Veterinary Medicine, Knoxville, TN, <i>Email: beckpen@utk.edu</i> .
114P	Identification of a novel herpesvirus DNA sequence in cutaneous ulcer lesions found in a sudden death case of a fisher (<i>Martes pennanti</i>).	N. Music, D. Tremblay, C. Gagnon, Faculté de médecine vétérinaire, Université de Montréal, Saint-Hyacinthe, QC, Canada; J. Tremblay, Zoo sauvage de St-Félicien, St-Félicien, QC, Canada; D. Larochelle, Laboratoire d'expertise en pathologie animale du Québec (LEPAQ) du Ministère de l'agriculture des pêcheries et de l'alimentation du Québec (MAPAQ), Ste-Foy, QC, Canada, <i>Email: nedzad.music@umontreal.ca</i> .
115P	Transcript expression analysis in tracheobronchial lymph nodes of pseudorabies virus infected pigs	L. Miller, D. Bayles, K. Lager, USDA-ARS-NADC, Ames, IA; G. Harhay, USDA-ARS-USMARC, Clay Center, NE; E. Zanella, Universidade de Passo Fundo, Passo Fundo, Brazil, <i>Email: laura.miller@ars.usda.gov</i> .
116P	The search of new ways of eradication of herpesvirus infections.	O. Zoz, Z. Klestova, Institute of Veterinary Medicine of NAASU, Kiev, Ukraine, <i>Email: zoz_olga@mail.ru</i> .
117P	Antibody response of pigs to the E protein of Porcine Reproductive and Respiratory Syndrome Virus.	M. Laoye, R. Vemulapalli, R. Pogranichniy, S. Lenz, D. Ragland, Purdue University, West Lafayette, IN, <i>Email: mlaoye@purdue.edu</i> .
118P	Three amino acids of ORF5 are jointly responsible for virulence of porcine reproductive and respiratory syndrome virus.	B. Kwon, H. Vu, L. Beura, S. Subramaniam, A. Pattnaik, F. Osorio, UNL, Lincoln, NE, <i>Email: bjkwon66@hotmail.com</i> .
119P	Interaction of PRRSV NSP1 β with the cellular poly (C)-binding proteins (PCBPs).	L. BEURA, A. Pattnaik, F. Osorio, UNIV. OF NEBRASKA, LINCOLN, LINCOLN, NE, <i>Email: lalitvet4098@gmail.com</i> .
120P	Interaction of PRRSV Nsp1 α and protein inhibitor of activated STAT1 (PIAS1) mediates sumoylation of Nsp1 α .	C. Song, Y. Du, O. Kim, D. Yoo, University of Illinois at Urbana-Champaign, Urbana, IL; H. Liu, North Carolina State University, Raleigh, NC, <i>Email: chsong@illinois.edu</i> .
121P	PRRSV Nsp1 beta subunit-based inhibition of interferon-beta production and signal transduction.	C. Song, D. Yoo, University of Illinois at Urbana-Champaign, Urbana, IL; P. Krell, University of Guelph, Guelph, ON, Canada, <i>Email: chsong@illinois.edu</i> .
122P	Efficacy of PRRSV vaccines against homologous and heterologous strain challenge	J. Abrahante, M. Murtaugh, University of Minnesota, St Paul, MN; M. Wagner, Fairmont Veterinary Clinic, Fairmont, MN, <i>Email: abrah023@umn.edu</i> .

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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
123P	Development of highly pathogenic Avian Influenza and Newcastle Disease molecular detection and differentiation techniques.	D. Muzyka, A. Gerilovych, B. Stegny, A. Stegny, V. Bolotin, NSC Institute of experimental and clinical veterinary medicine, Kharkiv, Ukraine, <i>Email: antger@rambler.ru</i> .
124P	Serological supervisions of avian influenza among wild birds in Ukraine in 2000 - 2009.	D. Muzyka, B. Stegny, NSC Institute of experimental and clinical veterinary medicine, Kharkiv, Ukraine, <i>Email: dmuzyka77@gmail.com</i> .
125P	Identification of a recombinant porcine norovirus and a porcine sapovirus of a new genogroup in Korea.	I. Choi, Y. Song, H. Nam, H. Bak, J. Lee, S. Park, C. Song, Konkuk University, College of Veterinary Medicine, Seoul, Korea, Republic of, <i>Email: ischoi@konkuk.ac.kr</i> .
126P	Fetal immunological effects and liver tolerance following persistent bovine viral diarrhea virus infection.	S. Morarie, J. Mediger, L. Braun, C. Chase, South Dakota State University, Sioux Falls, SD; N. Smirnova, T. Hansen, Colorado State University, Fort Collins, CO, <i>Email: semorarie@jacks.sdstate.edu</i> .
127P	Clinical characterization of Bovine Viral Diarrhea Virus from Korean indigenous calves.	K. Choi, M. Song, Kyungpook National University, Sangju, Korea, Republic of, <i>Email: kschoi3@knu.ac.kr</i> .

ORAL PROGRAM

BACTERIAL PATHOGENESIS

Monday, Dec. 6, 2010 - Avenue Ballroom - 4th Floor

Section Leaders: Jun Lin and Gireesh Rajashekara

Oral presentations 15 minutes: 10-12 minutes, 3-5 minutes interactive discussion.

Keynote presentations: 35-40 minute oral presentation and a 5-10 minute interactive discussion.

Time	No.	Title	Authors
		Presiders: Jun Lin and Raul Almeida	
8:00 Mon.	001	Mastitis in selected areas in the Philippines: prevalence, etiology, and antibiotic sensitivity profile	M. Gordoncillo, P. Bartlett, College of Veterinary Medicine, East Lansing, MI; J. Bautista, I. Sarmago, Dairy Research and Training Institute, University of the Philippines, Los Banos, Laguna, Philippines; M. Hikiba, JOCV/JICA-DTRI-NDA-PNVSCA Dairy Development Enhancement Project, Laguna, Philippines; J. Haguigan, J. Acuna, University of the Philippines, Los Banos, Laguna, Philippines, <i>Email: gordonci@cvm.msu.edu</i> .
8:15	002	Identification of <i>Staphylococcus aureus</i> genes highly expressed during bovine mastitis: A unique opportunity for vaccine development.	C. Ster, M. Allard, S. Boulanger, B. Talbot, F. Malouin, Université de Sherbrooke, Sherbrooke, QC, Canada; P. Lacasse, Agriculture and Agri Food Canada, Sherbrooke, QC, Canada, <i>Email: celine.ster@usherbrooke.ca</i> .
8:30	003	Bovine mammary endothelial cell inflammatory responses after in vitro <i>Streptococcus uberis</i> challenge.	G. Contreras, C. Corl, E. Naplin, J. Gandy, L. Sordillo, Michigan State University, East Lansing, MI, <i>Email: contrera@cvm.msu.edu</i> .
8:45	004	MAP suppression of caspase activity in infected primary bovine macrophages	E. Kabara, P. Coussens, Michigan State University, East Lansing, MI, <i>Email: kabaraed@msu.edu</i> .
9:00	005	Brucellosis in sheep & goats of Bogra and Mymensingh Districts of Bangladesh.	M. Rahman, M. Mahasin, N. Jahan, Bangladesh Agricultural University, Mymensingh, Bangladesh, <i>Email: prithul02@yahoo.co.uk</i> .
9:15	006	Identification of enhanced pathogenicity of <i>Brachyspira</i> species associated with clinical disease in swine.	K. Clothier, J. Kinyon, T. Frana, E. Strait, N. Naberhaus, L. Bower, A. Chriswell, K. Schwartz, Iowa State University, Ames, IA, <i>Email: clothier@iastate.edu</i> .
9:30		Break and Table Top Exhibits – Foyer	
		Presiders: Jun Lin and Gireesh Rajashekara	
9:45 Mon.	007	Functional analysis of methyl accepting chemotaxis proteins in <i>Campylobacter jejuni</i> .	D. Gangaiah, K. Chandrashekar, G. Rajashekara, The Ohio State University, Wooster, OH, <i>Email: gangaiah.1@osu.edu</i> .
10:00	008	The ferric uptake regulator (fur) and iron uptake of <i>Edwardsiella ictaluri</i> .	J. Santander, R. Curtiss, Arizona State University, Tempe, AZ, <i>Email: jasantander@asu.edu</i> .
10:15	009	Identification of a novel <i>Campylobacter</i> enterobactin esterase cee by whole genome sequencing and comparative genomic analysis	X. Zeng, F. Xu, J. Lin, University of Tennessee, Knoxville, TN; B. Jeon, University of Prince Edward Island, Charlottetown, PE, Canada; Q. Zhang, Iowa State University, Ames, IA, <i>Email: xzeng3@utk.edu</i> .

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BACTERIAL PATHOGENESIS

Monday, Dec. 6, 2010 - Avenue Ballroom - 4th Floor

Section Leaders: Jun Lin and Gireesh Rajashekara

Time	No.	Title	Authors
10:30 Mon.	010	Identification of a high affinity adhesin of <i>Fusobacterium necrophorum</i> subsp. <i>necrophorum</i> that mediates binding to Bovine Endothelial Cells.	A. Kumar, T. Nagaraja, S. Narayanan, Kansas State University, Manhattan, KS, <i>Email: akumar@vet.ksu.edu</i> .
10:45-11:30 Keynote	011	Interplay of <i>M. tuberculosis</i> and macrophages in the human respiratory system.	Larry Schlesinger. Division of Infectious Diseases and the Center for Microbial Interface Biology, The Ohio State University, Columbus, OH
11:30		Lunch and Table Top Exhibits - Foyer	
		Presiders: Gireesh Rajashekara and Raul Almeida	
1:30 Mon.	012	Reinfection of mice with <i>Listeria monocytogenes</i> during pregnancy is associated with inflammation in murine fetoplacental units despite a decreased microbial load.	K. Poulsen, N. Faith, H. Steinberg, C. Czuprynski, University of Wisconsin-Madison, Madison, WI, <i>Email: poulsenk@svm.vetmed.wisc.edu</i> .
1:45	013	Identification of genes required for <i>Campylobacter</i> resistance to fowlicidin-1, a chicken host defense peptide.	K. Hoang, J. Lin, The University of Tennessee, Knoxville, TN, <i>Email: vhoang1@utk.edu</i> .
2:00	014	Causes of failure on breeding soundness examination in Western U.S. Rams (2000-2007).	P. Morley, D. Van Metre, S. Rao, Colorado State University, Fort Collins, CO, <i>Email: paul.morley@colostate.edu</i> .
2:15	015	Characterization of β -lactam resistance determinants in <i>Escherichia Coli</i> and <i>Klebsiella</i> Spp. from bovine mastitis in Canada.	S. Nilsson, P. Boerlin, University of Guelph, Guelph, ON, Canada; J. McClure, University of Prince Edward Island, Charlottetown, PE, Canada, <i>Email: nilssons@uoguelph.ca</i> .
2:30	016	Class 1 integrons and integrase gene in <i>Escherichia coli</i> isolated from Canadian beef cattle.	M. Leslie, P. Boerlin, University of Guelph, Guelph, ON, Canada; T. Alexander, T. McAllister, Agriculture and Agri-Food Canada Research Centre, Lethbridge, AB, Canada; S. Gow, Laboratory for Foodborne Zoonoses, Saskatoon, SK, Canada; R. Reid-Smith, Laboratory for Foodborne Zoonoses, Guelph, ON, Canada. <i>Email: lesliem@uoguelph.ca</i> .
2:45		Break and Table Top Exhibits – Foyer	
		Presiders: Jun Lin and Gireesh Rajashekara	
3:00 Mon.	017	<i>Staphylococcus aureus</i> enhanced intracellular survival through coagulation manipulation.	R. Ortiz Marty, W. Wark, I. Mullarky, Virginia Tech, Blacksburg, VA, <i>Email: rjortiz@vt.edu</i> .
3:15	018	Microbiological and molecular characterization of coagulase positive <i>Staphylococcus</i> species isolated from canine clinical specimens.	D. Diaz-Campos, T. Hathcock, R. Palomares-Naveda, K. Brock, Auburn University, Auburn, AL, <i>Email: diazcam@auburn.edu</i> .

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BACTERIAL PATHOGENESIS

Monday, Dec. 6, 2010 - Avenue Ballroom - 4th Floor

Section Leaders: Jun Lin and Gireesh Rajashekara

Time	No.	Title	Authors
3:30 Mon.	019	The complete genome of <i>Erysipelothrix rhusiopathiae</i> , a causative agent of swine erysipelas.	Y. Shimoji, Y. Ogawa, F. Shi, National Institute of Animal Health, Tsukuba, Ibaraki, Japan; K. Kurokawa, Tokyo Institute of Technology, Tokyo, Japan; T. Hayashi, University of Miyazaki, Miyazaki, Japan, <i>Email: shimoji@affrc.go.jp</i> .
3:45	020	High throughput transcript mapping of <i>Histophilus somni</i> 2336 by deep sequencing	Ranjit Kumar ¹ , Bindu Nanduri ¹ , James Watt ² Amanda Cooksey ³ , and Mark L. Lawrence ¹ ¹ College of Veterinary Medicine, Mississippi State University, Mississippi State, MS. ² U. Air Force Medical Center, Keesler Air Force Base, Biloxi, MS. ³ Life Sciences and Biotechnology Institute, Mississippi State University, Mississippi State, MS.
4:00	021	GenHtr_Quan a quantitative approach for comparative variant analysis of bacterial pathogens.	G. Yu, Boise State University, Boise, ID, <i>Email: gongxinyu@boisestate.edu</i> .
4:15 Mon.	022	Profiling of porcine tonsil bacterial communities using Terminal Restriction Fragment Length Polymorphism (T-RFLP)	S. Ojha, D. Slavic, S. Chen, Z. Poljak, J. MacInnes, University of Guelph, Guelph, ON, Canada, <i>Email: macinnes@uoguelph.ca</i> .
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

Monday, Dec. 6, 2010 - Denver/Houston Room - 5th Floor

Section Leaders: Scott Dee and Gabriele Landolt

Oral presentations 15 minutes: 10-12 minutes, 3-5 minutes interactive discussion.

Keynote presentations: 35-40 minute oral presentation and a 5-10 minute interactive discussion.

Time	No.	Title	Authors
11:30		Lunch and Table Top Exhibits - Foyer	
1:30 Mon.	023	Effect of biosecurity, movement restrictions, and vaccination in the control of foot and mouth disease in livestock production systems in the central United States.	M. Sanderson, Kansas State University, Manhattan, KS; K. Forde-Folle, USDA:APHIS:VS:CEAH, Ft Collins, CO; A. Reeves, Colorado State University, Ft Collins, CO, <i>Email: sandersn@vet.k-state.edu</i> .
1:45	024	Effect of vaccination strategy in the control of foot and mouth disease in livestock production systems in the central United States.	M. Sanderson, Kansas State University, Manhattan, KS; K. Forde-Folle, USDA:APHIS:VS:CEAH, Ft Collins, CO; A. Reeves, Colorado State University, Ft Collins, CO, <i>Email: sandersn@vet.k-state.edu</i> .
2:00	025	Production region model of PRRSV and <i>Mycoplasma hyopneumoniae</i> transmission and biosecurity: Results of the 3-year study.	S. Otake, S. Dee, A. Pitkin, J. Deen, University of Minnesota, St. Paul, MN, <i>Email: otak0001@umn.edu</i> .
2:15	026	Prevention of PRRSV infection in large breeding swine herds located in swine dense regions by using air filtration: Preliminary observations	S. Dee, S. Otake, University of Minnesota, St. Paul, MN; G. Spronk, Pipestone Veterinary Clinic, Pipestone, MN, <i>Email: deexx004@umn.edu</i> .
2:30	027	Evaluation of a needle-free injection system (AcuShot™) for reduction of hematogenous transmission of PRRS virus.	S. Baker, University of Minnesota, Saint Paul, MN; E. Mondaca, D. Polson, Boehringer Ingelheim Vetmedica, Inc, Saint Joseph, MO; S. Dee, College of Veterinary Medicine, University of Minnesota, Saint Paul, MN, <i>Email: baker115@umn.edu</i> .
2:45		Break and Table Top Exhibits – Foyer	
3:00-3:45 Keynote	028	Beyond normal science: paradigm shifts in veterinary infection control.	Paul S. Morley, James L. Voss Veterinary Teaching Hospital, Colorado State University, Fort Collins, CO
3:45	029	Evaluating Porcine Circovirus Type 2 control in vaccinated herds: are sentinels needed?	C. Venegas-Vargas, B. Straw, Michigan State University, East Lansing, MI, <i>Email: venegasv@cvm.msu.edu</i> .
4:00 Mon.	030	Methicillin resistant <i>Staphylococcus</i> spp in commercial pigs used in veterinary student training.	B. Burgess, D. Czerniak, M. Jacobsson, D. Bolte, P. Morley, Colorado State University, Fort Collins, CO; J. Weese, University of Guelph, Guelph, ON, Canada, <i>Email: brandy.burgess@colostate.edu</i> .
4:30 to 5:00	5:00	Break and Table Top Exhibits – Foyer	
5:00 to 6:30	6:30	Poster Session II Grand Ballroom Salon III - 7th floor	

COMPANION ANIMAL EPIDEMIOLOGY

Tuesday, Dec. 7, 2010 - Avenue Ballroom - 4th Floor

Section Leader: Laura Hungerford

Oral presentations 15 minutes: 10-12 minutes, 3-5 minutes interactive discussion.

Keynote presentations: 35-40 minute oral presentation and a 5-10 minute interactive discussion.

Time	No.	Title	Authors
8:00 Tues.	031	Zoonotic disease awareness in animal shelter workers and volunteers and the effect of training.	K. Steneroden, A. Hill, M. Salman, Colorado State University, Fort Collins, CO, <i>Email: kksten@colostate.edu</i> .
8:15	032	Comparison of antimicrobial resistance patterns of <i>Salmonella</i> spp. and generic <i>E. coli</i> recovered from pet dogs from volunteer households in Ontario, Canada (2005-2006).	E. Leonard, D. Pearl, N. Janecko, J. Weese, A. Peregrine, University of Guelph, Guelph, ON, Canada; R. Finley, R. Reid-Smith, University of Guelph and Public Health Agency of Canada, Guelph, ON, Canada, <i>Email: eleonard@uoguelph.ca</i> .
8:30	033	Seroprevalence of canine influenza virus (H3N8) in racing sled dogs.	H. Pecoraro, J. Lee, G. Landolt, Colorado State University, Fort Collins, CO, <i>Email: hlp@lamar.colostate.edu</i> .
8:45	034	Practicality, feasibility, and validity of capture-recapture method to estimate dog population in Lumlukka District, Pathumthani Province, Thailand.	V. Wongphruksasoong, Ministry of Agriculture and Cooperation, Bangkok, Thailand, <i>Email: lhin_001@hotmail.com</i> .
9:00	035	Prevalence of <i>Giardia</i> spp. and <i>Cryptosporidium</i> spp. in dogs in Chiang Mai, Thailand.	S. Tangtrongsup, A. Scorza, M. Lappin, M. Salman, Department of Clinical Sciences, College of Veterinary Medicine and Biomedical Sciences, Colorado State University, Fort Collins, CO, <i>Email: sahatchai.tangtrongsup@colostate.edu</i> .
9:15	036	Results of an international survey of Otterhound health.	K. Evans, Animal Health Trust, Newmarket, United Kingdom; V. Adams, Veterinary Epidemiology Consultant, Barton Mills, United Kingdom, <i>Email: katy.evans@aht.org.uk</i> .
9:30		Break and Table Top Exhibits – Foyer	
10:00 Tues.	037	Results of the 2004 KC/BSAVA purebred dog health survey: Caesarean section rates for UK pedigree dogs.	V. Adams, Veterinary Epidemiology Consultant, Barton Mills, United Kingdom; K. Evans, Animal Health Trust, Kentford, United Kingdom, <i>Email: vetepi@vickijadams.co.uk</i> .
10:15	038	Results of the 2004 UK KC/BSAVA purebred dog health survey: Mortality and morbidity due to gastric dilatation-volvulus syndrome.	V. Adams, Veterinary Epidemiology Consultant, Barton Mills, United Kingdom; K. Evans, Animal Health Trust, Kentford, United Kingdom, <i>Email: vetepi@vickijadams.co.uk</i> .
10:30	039	Clustering of serologically diagnosed cases of Coccidioidomycosis among dogs in Texas.	R. Gautam, I. Srinath, M. Bani-Yaghoub, A. Clavijo, R. Ivanek, Texas A&M, College Station, TX, <i>Email: rgautam@cvm.tamu.edu</i> .
10:45	040	Evaluation of collars and microchips for visual and permanent identification of pet cats	L. Lord, The Ohio State University, Columbus, OH; B. Griffin, J. Levy, University of Florida, Gainesville, FL; M. Slater, American Society for the Prevention of Cruelty to Animals, Urbana, IL, <i>Email: lord.19@osu.edu</i> .

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COMPANION ANIMAL EPIDEMIOLOGY
Tuesday, Dec. 7, 2010 - Avenue Ballroom - 4th Floor
Section Leader: Laura Hungerford

Time	No.	Title	Authors
11:00 Tues.	041	Characterization of advertisements for puppies sold online: Determinants of cost and a comparison with parent club breeders	H. Voris, L. Lord, T. Wittum, P. Rajala-Schultz, The Ohio State University, Columbus, OH, <i>Email: lord.19@osu.edu</i> .
11:15	042	Using number-needed-to-treat to quantify risk in the context of breed-specific legislation and public safety.	M. Slater, The American Society for the Prevention of Cruelty to Animals, Urbana, IL; G. Patronek, A. Marder, Animal Rescue League of Boston, Boston, MA, <i>Email: margaretslater@aspca.org</i> .
11:45 to	12:30	Business Meeting: Dedication, New Members Introduction, and Graduate Student Competition Awards Presentations	

EPIDEMIOLOGY AND ANIMAL HEALTH ECONOMICS

Monday, Dec. 6, 2010 - Salons A/B/C/D - 5th Floor

Section Leader: Ashley Hill

Oral presentations 15 minutes: 10-12 minutes, 3-5 minutes interactive discussion.

Keynote presentations: 35-40 minute oral presentation and a 5-10 minute interactive discussion.

Time	No.	Title	Authors
8:00-8:45 Mon. Keynote	075	Food Systems Veterinary Medicine for the 21st Century.	H. S. Hurd, Department of Veterinary Diagnostic and Production Animal Medicine, College of Veterinary Medicine, Iowa State University, Ames IA.
8:45	043	Risk factors associated with carriage of <i>Clostridium difficile</i> in horses admitted to a veterinary teaching hospital in Atlantic Canada.	L. Connor, M. Saab, C. McClure, J. McClure, Department of Health Management, Atlantic Veterinary College, Charlottetown, PE, Canada, <i>Email: jmcclure@upei.ca</i> .
9:00	044	An epidemiologic analysis of <i>Salmonella</i> spp shedding in hospitalized horses with or without diarrhea.	A. Ekiri, A. Morton, M. Long, T. Krueger, R. MacKay, J. Hernandez, University of Florida, Gainesville, FL, <i>Email: ekiria@ufl.edu</i> .
9:15	045	Purchase price, exercise history, lameness, speed and sales price in 2-year-old in-training Thoroughbred horses.	S. Preston, D. Zimmel, T. Chmielewski, A. Morton, M. Brown, J. Hernandez, University of Florida, Gainesville, FL; T. Trumble, University of Minnesota, St. Paul, MN, <i>Email: sadver98@aol.com</i> .
9:30		Break and Table Top Exhibits – Foyer	
10:00	046	Clustering of and Risk Factors for the Porcine High Fever Disease in a Region of Vietnam	H. Le, Z. Poljak, C. Dewey, R. Deardon, University of Guelph, Guelph, ON, Canada, <i>Email: zpoljak@uoguelph.ca</i> .
10:15	047	Serological prevalence of Japanese encephalitis virus in pigs in four mountain districts in Nepal.	K. Thakur, A. Johnson, Purdue University, West Lafayette, IN; G. Pant, Department of Livestock Services, Central Veterinary Laboratory, Kathmandu, Nepal; R. Pogranichniy, Purdue University / Animal Disease Diagnostic Laboratory, West Lafayette, IN, <i>Email: kthakur@purdue.edu</i> .
10:30	048	The association between submission counts to a veterinary diagnostic laboratory and the economic and disease challenges of the Ontario swine industry from 1998-2009.	T. O'Sullivan, R. Friendship, D. Pearl, B. McEwen, A. Ker, C. Dewey, University of Guelph, Guelph, ON, Canada, <i>Email: tosulliv@uoguelph.ca</i> .
10:45	049	Molecular epidemiology of porcine reproductive and respiratory syndrome virus (PRRSV) in Ontario, Canada.	M. Brar, M. Shi, F. Leung, University of Hong Kong, Hong Kong, China; S. Carman, University of Guelph, Guelph, ON, Canada; M. Murtaugh, University of Minnesota, Minnesota, MN, <i>Email: manreet@hku.hk</i> .
11:00	050	One year's study of dynamic and evolution of type I and II PRRSV in a seed-stock farm	H. Kim, S. Park, S. Rho, J. Han, V. Nguyen, B. Park, Seoul National University, Seoul, Korea, Republic of, <i>Email: khk1329@snu.ac.kr</i> .
11:15 Mon.	051	Pen-based oral fluid sampling for PRRSV in low prevalence situations.	J. Prickett, R. Main, W. Chittick, J. Zimmerman, Iowa State University, Ames, IA; M. Hoogland, C. Rademacher, Murphy-Brown, L.L.C., Ames, IA, <i>Email: prickett@iastate.edu</i> .

EPIDEMIOLOGY AND ANIMAL HEALTH ECONOMICS

Monday, Dec. 6, 2010 - Salons A/B/C/D - 5th Floor

Section Leader: Ashley Hill

Time	No.	Title	Authors
11:30		Lunch and Table Top Exhibits - Foyer	
1:30 Mon.	052	Accuracy of disk diffusion and broth microdilution by latent class analysis for <i>Escherichia Coli</i> and <i>Mannheimia Haemolytica</i> in feedlot cattle.	K. Benedict, P. Morley, Colorado State University, Fort Collins, CO; S. Gow, Public Health Agency of Canada, Saskatoon, SK, Canada; R. Reid-Smith, Public Health Agency of Canada, Guelph, ON, Canada; C. Booker, Feedlot Health Management Services, Okotoks, AB, Canada; T. McAllister, University of Lethbridge, Lethbridge, AB, Canada, <i>Email: kbened@colostate.edu</i> .
1:45	053	Validity of the bovine TB gamma interferon assay on blood collected during exsanguination at slaughter.	C. Okafor, D. Grooms, S. Bolin, J. Kaneene, Michigan State University, East Lansing, MI, <i>Email: okaforch@cvm.msu.edu</i> .
2:00	054	A Bayesian approach to estimate test parameters of 2 different tests for detection of <i>Mycobacterium avium</i> subsp. <i>paratuberculosis</i> fecal shedding in dairy cows.	L. Espejo, S. Wells, University of Minnesota, Saint Paul, MN; F. Zagmutt, H. Groenendaal, Vose Consulting, Boulder, CO, <i>Email: espe0048@umn.edu</i> .
2:15	055	Estimates of diagnostic test sensitivities and specificities: what confidence do we really have?	S. Guillosoy, H. Scott, J. Richt, College of Veterinary Medicine, Kansas State University, Manhattan, KS, <i>Email: sguill@vet.k-state.edu</i> .
2:30	056	Comparison of crude and model-adjusted space-time scan statistic for food animal syndromic surveillance.	G. Alton, D. Pearl, K. Bateman, O. Berke, University of Guelph, Guelph, ON, Canada; W. McNab, Ontario Ministry of Agriculture, Food and Rural Affairs, Guelph, ON, Canada, <i>Email: altong@uoguelph.ca</i> .
2:45		Break and Table Top Exhibits – Foyer	
3:00 Mon.	057	Assessment of passive laboratory data for use in an antimicrobial resistance surveillance system.	S. Glass-Kaasta, D. Pearl, R. Reid-Smith, B. McEwen, D. Slavic, S. McEwen, J. Fairles, University of Guelph, Guelph, ON, Canada; J. Parmley, D. Leger, A. Agunos, Public Health Agency of Canada, Guelph, ON, Canada, <i>Email: sglass@uoguelph.ca</i> .
3:15	058	Development of a longitudinal antimicrobial resistance and antimicrobial use surveillance program for the feedlot sector in Canada: Lessons Learned.	S. Gow, Public Health Agency of Canada, Canadian Integrated Program for Antimicrobial Resistance Surveillance, Saskatoon, SK, Canada; S. Checkley, University of Calgary, Department of Ecosystem and Public Health, Calgary, AB, Canada; C. Booker, Feedlot Health Management Services Ltd., Okotoks, AB, Canada; T. McAllister, Lethbridge Research Centre, Agriculture and Agri-Food Canada, Lethbridge, AB, Canada; P. Boerlin, Ontario Veterinary College, Department of Pathobiology, Calgary, AB, Canada; R. Read, University of Calgary, Faculty of Medicine, Health Sciences Centre,, Calgary, AB, Canada; P. Morley, Colorado State University, Animal Population Health Institute, Fort Collins, CO, <i>Email: sheryl.gow@usask.ca</i> .

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EPIDEMIOLOGY AND ANIMAL HEALTH ECONOMICS

Monday, Dec. 6, 2010 - Salons A/B/C/D - 5th Floor

Section Leader: Ashley Hill

Time	No.	Title	Authors
3:30 Mon.	059	Measurement of low-quantity antibiotic resistance genes in agricultural samples: a hierarchical model for analysis of left-censored qPCR data.	T. Boyer, University of Minnesota, Minneapolis, MN; R. Singer, University of Minnesota, Saint Paul, MN, <i>Email: boye0087@umn.edu</i> .
3:45	060	Role of the environment in transmission of multi drug resistant (MDR) <i>Campylobacter</i> in antimicrobial free (ABF) pigs from farm to slaughter.	M. Quintana-Hayashi, H. Pierce, S. Thakur, North Carolina State University, Raleigh, NC, <i>Email: mpquinta@ncsu.edu</i> .
4:00	061	The human-animal Brucellosis interface in Republic of Georgia: A descriptive study using a rapid assessment approach.	K. Havas, M. Salman, Colorado State University, Fort Collins, CO; M. Ramishvili, A. Navdarashvili, Department of Anthrax, Zoonotic and Anaerobic Infections, National Center for Disease Control and Public Health of Georgia, Tbilisi, Georgia, <i>Email: karyn.havas@gmail.com</i> .
4:15 Mon.	062	Bovine tuberculosis slaughter surveillance system in the USA and the ability to trace infected cattle back to the herd of origin.	H. Mann, M. Salman, F. Olea-Popelka, Department of Clinical Sciences, College of Veterinary Medicine and Biomedical Sciences, Colorado State University, Animal Population Health Institute, Fort Collins, CO; K. Orloski, United States Department of Agriculture, Animal and Plant Health Inspection Service, Veterinary Services, Fort Collins, CO; R. Basaraba, Department of Microbiology, Immunology and Pathology, College of Veterinary Medicine and Biomedical Sciences, Colorado State University, Fort Collins, CO, <i>Email: Hmann1978@gmail.com</i> .
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	

EPIDEMIOLOGY AND ANIMAL HEALTH ECONOMICS

Tuesday, Dec. 7, 2010 - Salons A/B/C/D - 5th Floor

Section Leader: Ashley Hill

Time	No.	Title	Authors
8:00 Tues.	063	A meta-analysis of the effects of feeding yeast culture produced by anaerobic fermentation of <i>Saccharomyces cerevisiae</i> , on milk production in lactating dairy cows.	G. Poppy, P. Morley, Colorado State University, Fort Collins, CO; W. Sanchez, K. Dorton, Diamond V, Cedar Rapids, IA; A. Rabie, I. Lean, SBScibus, Camden, Australia, <i>Email: drgpoppy@gmail.com</i> .
8:15	064	Correspondence analysis-a method of assessing relationships between categorical variables.	C. Haley, USDA:APHIS:VS:CEAH:NAHMS, Fort Collins, CO; D. Van Metre, Department of Clinical Sciences, Colorado State University, Fort Collins, CO, <i>Email: Charles.A.Haley@aphis.usda.gov</i> .
8:30	065	Using mixed treatment comparisons meta-analysis to compare interventions for which no head-to-head comparison exists: Treatments for bovine respiratory disease.	A. O'Connor, Iowa State University, Ames, IA, <i>Email: oconnor@iastate.edu</i> .
8:45	066	Population level inference of antimicrobial resistance genotype from phenotypic data using Bayesian MCMC.	M. Denwood, A. Mather, D. Haydon, L. Matthews, D. Mellor, S. Reid, University of Glasgow, Glasgow, United Kingdom, <i>Email: Matthew.Denwood@glasgow.ac.uk</i> .
9:00	067	Modelling of PD dynamics in post-smolt farmed Atlantic salmon.	S. Tavoranpanich, H. Viljugrein, P. Jansen, E. Brun, National Veterinary Institute, Oslo, Norway; A. Stene, University of Ålesund, Ålesund, Norway, <i>Email: saraya.tavoranpanich@vetinst.no</i> .
9:15	068	Diagnostic sampling strategies for virulent ovine footrot: simulating detection of <i>Dichelobacter nodosus</i> serogroups for bivalent vaccine formulation	A. Hill, Colorado State University, Fort Collins, CO, CO; O. Dhungyel, R. Whittington, University of Sydney, Camden, NSW, Australia, <i>Email: Ashley.Hill@colostate.edu</i> .
9:30		Break and Table Top Exhibits – Foyer	
10:00	069	Contributions to ecological diversity of antimicrobial resistance on dairy farms	A. Mather, S. Reid, D. Haydon, D. Mellor, University of Glasgow, Glasgow, United Kingdom; W. Sisco, Washington State University, Pullman, WA, <i>Email: a.mather@vet.gla.ac.uk</i> .
10:15 Tues.	070	Retrospective study of HPAI outbreaks in domestic poultry in mainland China from 2004 to 2009.	Y. Wang, B. Huang, China Animal Health and Epidemiology Centre, Qingdao city, China; K. Wongsathapornchai, Bureau of Disease Control and Veterinary Services, Department of Livestock Development, Ministry of Agriculture and Cooperatives, Bangkok, Thailand; V. Martin, Emergency Center for Transboundary Animal Diseases, Food and Agriculture Organization of the United Nations, Beijing, China, <i>Email: wangym@msn.com</i> .

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EPIDEMIOLOGY AND ANIMAL HEALTH ECONOMICS

Tuesday, Dec. 7, 2010 - Salons A/B/C/D - 5th Floor

Section Leader: Ashley Hill

Time	No.	Title	Authors
10:30 Tues.	071	Public health and zoonoses: A survey of Canadian Public Health personnel regarding knowledge, practice and education.	K. Snedeker, M. Anderson, J. Weese, J. Sargeant, Ontario Veterinary College, Guelph, ON, Canada, <i>Email: snedeker@uoguelph.ca</i> .
10:45	072	Carriage of methicillin-resistant <i>Staphylococcus aureus</i> (MRSA) among selected Michigan 4-H club members and their backyard-raised pigs	M. Gordoncillo, P. Bartlett, College of Veterinary Medicine, East Lansing, MI; N. Abdujamilova, National Food Safety and Toxicology Center, Michigan State University, East Lansing, MI; M. Perri, S. Donabedian, M. Zervos, Henry Ford Hospital, Detroit, MI, <i>Email: gordonci@cvm.msu.edu</i> .
11:00	073	Environmental role in the transmission of multidrug resistant (MDR) Salmonella to conventional pigs at different stage of production at farm and slaughter.	S. Keelara Veerappa, S. Thakur, North Carolina State University, Raleigh, NC, <i>Email: skeelar@ncsu.edu</i> .
11:15 Tues.	074	Prevalence of zoonotic pathogens in the Canadian pork chain: evidence from a scoping review.	S. Parker, University of Saskatchewan, Saskatoon, SK, Canada; A. Rajić, B. Wilhelm, A. Fazil, Laboratory for Foodborne Zoonoses, Public Health Agency of Canada, Guelph, ON, Canada; S. McEwen, University of Guelph, Guelph, ON, Canada, <i>Email: sarah.parker@usask.ca</i> .
11:45 to	12:30	Business Meeting: Dedication, New Members Introduction, and Graduate Student Competition Awards Presentations	

FOOD AND ENVIRONMENTAL SAFETY
Monday, Dec. 6, 2010 - Salon E - 5th Floor
Section Leader: Yvette Johnson

Oral presentations 15 minutes: 10-12 minutes, 3-5 minutes interactive discussion.

Keynote presentations: 35-40 minute oral presentation and a 5-10 minute interactive discussion.

Time	No.	Title	Authors
8:00-8:45 Mon. Keynote	075	Food Systems Veterinary Medicine for the 21st Century.	H. S. Hurd, Department of Veterinary Diagnostic and Production Animal Medicine, College of Veterinary Medicine, Iowa State University, Ames IA.
8:45	076	Comparison of <i>Salmonella</i> testing results among NAHMS Dairy Studies conducted in 2007, 2002, and 1996.	C. Fossler, J. Lombard, D. Dargatz, USDA:APHIS:VS, Fort Collins, CO, <i>Email: charles.p.fossler@aphis.usda.gov</i> .
9:00	077	Dose- and serotype- dependent dynamics of fecal shedding and immune response post <i>Salmonella</i> inoculation in pigs.	R. Ivanek, Texas A&M University, College Station, TX; J. Österberg, S. Sternberg Lewerin, National Veterinary Institute, Uppsala, Sweden, <i>Email: rivanek@cvm.tamu.edu</i> .
9:15	078	Molecular characterization of multidrug resistant (MDR) <i>Salmonella</i> Typhimurium from swine and human origin by phage types, resistance genes, integrons and PFGE.	S. Keelara Veerappa, S. Thakur, North Carolina State University, Raleigh, NC, <i>Email: skeelar@ncsu.edu</i> .
9:30		Break and Table Top Exhibits – Foyer	
10:00	079	Longitudinal study of Salmonella shedding in finishing pigs in a multi-site production system.	A. Pires, J. Funk, Department of Large Animal Clinical Sciences, College of Veterinary Medicine, Michigan State University, East Lansing, MI; R. Manuzon, L. Zhao, Department of Food, Agricultural and Biological Engineering, The Ohio State University, Columbus, OH, <i>Email: piresald@cvm.msu.edu</i> .
10:15	080	<i>Salmonella enterica</i> in swine production: Temporal and spatial diversity based on amplified fragment length polymorphism method.	B. Wang, J. McKean, A. O'Connor, Iowa State University, Ames, IA, <i>Email: wangbing810707@gmail.com</i> .
10:30	081	Variation in RAPD-PCR patterns is not attributable to genetic differences in <i>Salmonella</i> Enteritidis.	D. Mathis, M. Lee, R. Berghaus, J. Maurer, University of Georgia, Athens, GA, <i>Email: demathis@uga.edu</i> .
10:45	082	Class 1 integrons among multidrug resistant <i>Salmonella enterica</i> serovars from food animals, production environment and humans from two geographic locations.	K. Davies, B. Molla, M. Abley, W. Gebreyes, Ohio State University, Columbus, OH, <i>Email: gebreyes.1@osu.edu</i> .
11:00	083	<i>Salmonella</i> vaccination of broiler breeder pullets reduces Salmonella prevalences and loads in broiler chickens.	R. Berghaus, S. Thayer, C. Hofacre, University of Georgia, College of Veterinary Medicine, Athens, GA; J. Smith, Fieldale Farms Corp., Baldwin, GA, <i>Email: berghaus@uga.edu</i> .
11:15 Mon.	084	Effect of vegetable protein meal use in poultry feed on colonization and shedding of <i>Salmonella heidelberg</i> in broiler birds.	W. Alali, University of Georgia, Griffin, GA; C. Hofacre, A. Batal, University of Georgia, Athens, GA; G. Mathis, Southern Poultry Research, Athens, GA, <i>Email: walali@uga.edu</i> .

FOOD AND ENVIRONMENTAL SAFETY
Monday, Dec. 6, 2010 - Salon E - 5th Floor
Section Leader: Yvette Johnson

Time	No.	Title	Authors
11:30		Lunch and Table Top Exhibits - Foyer	
1:30 Mon.	085	Movement of Bacteria in the Environment via the Lesser Mealworm, <i>Alphitobius diaperinus</i> (Coleoptera: Tenebrionidae).	T. Crippen, C. Sheffield, US Dept Agriculture, College Station, TX; L. Zheng, Huazong Agricultural University, Wuhan, China, <i>Email: tc.crippen@ars.usda.gov</i> .
1:45	086	Characterization of the bacteriophage Felix O1 endolysin.	L. Settle, N. Sriranganathan, F. Pierson, Virginia Maryland Regional College of Veterinary Medicine, Blacksburg, VA; M. Seleem, Virginia Tech, Blacksburg, VA, <i>Email: lsettle@vt.edu</i> .
2:00	087	Absence of antimicrobial resistance in emerging Salmonella Newport isolates in Argentina from 2001-2007.	A. Mayer, R. Singer, University of Minnesota, St Paul, MN; M. Pichel, Instituto Nacional de Enfermedades Infecciosas – ANLIS "Carlos G. Malbrán", Buenos Aires, Argentina, <i>Email: mayer177@umn.edu</i> .
2:15	088	Development of a multiplex PCR for the detection of major serotypes of Shiga-toxin producing <i>E. coli</i> in bovine feces.	Z. Paddock, X. Shi, T. Nagaraja, J. Bai, Kansas State University, Manhattan, KS, <i>Email: zpaddock@ksu.edu</i> .
2:30	089	A multiplex, real-time PCR assay based on stx1, stx2, and rfbE for the quantification of <i>Escherichia coli</i> O157 in cattle feces.	M. Jacob, B. An, X. Shi, T. Nagaraja, J. Bai, Kansas State University, Manhattan, KS, <i>Email: mjacob@vet.k-state.edu</i> .
2:45		Break and Table Top Exhibits – Foyer	
3:00 Mon.	090	Impact of seasonal variation in ambient temperature on the transmission dynamics of a cattle pathogen: A modeling study of <i>Escherichia coli</i> O157:H7 infection in a dairy herd.	R. Gautam, M. Bani-Yaghoub, W. Neill, R. Ivanek, Texas A&M, College Station, TX; D. Dopfer, C. Kaspar, University of Wisconsin, Madison, WI, <i>Email: rgautam@cvm.tamu.edu</i> .
3:15	091	Effects of controlled intervention strategies on the quantities of a ceftiofur resistance gene (<i>bla</i> CMY-2) in the feces of feedlot cattle.	N. Kanwar, H. Scott, J. Vinasco, Kansas State University, Manhattan, KS; B. Norby, Texas A & M University, College Station, TX; S. Moore, West Texas A & M University, Canyon, TX; G. Loneragan, Texas Tech University, Lubbock, TX, <i>Email: neenak@vet.k-state.edu</i> .
3:30	092	A systematic review of vaccines to reduce the shedding of <i>E. coli</i> O157 in the feces of domestic ruminants.	K. Snedeker, M. Campbell, J. Sargeant, Ontario Veterinary College, Guelph, ON, Canada, <i>Email: snedeker@uoguelph.ca</i> .
3:45	093	Occurrence of Methicillin Resistant <i>Staphylococcus aureus</i> (MRSA) on-farm, at slaughter and retail pork in commercial swine and implications for food safety.	B. Molla, M. Byrne, M. Abley, W. Gebreyes, The Ohio State University, Columbus, OH; C. Jackson, USDA-ARS, Athens, GA; T. Smith, The University of Iowa, Iowa City, IA; P. Davies, University of Minnesota, St. Paul, MN, <i>Email: molla.7@osu.edu</i> .
4:00 Mon.	094	Prevalence and genotypic characterization of Staphylococcal isolates from Northeastern Brazil.	T. Gearhart, M. Abley, N. Tiao, V. Ponte, W. Gebreyes, The Ohio State University, Columbus, OH; W. Lopez, C. Oliveria, Centro de Ciências Agrárias (CCA/UFPB), Areia, Brazil, <i>Email: abley.1@osu.edu</i> .

FOOD AND ENVIRONMENTAL SAFETY
Monday, Dec. 6, 2010 - Salon E - 5th Floor
Section Leader: Yvette Johnson

Time	No.	Title	Authors
4:15 Mon.	095	Antimicrobial drug resistance among enterococci from broilers fed antimicrobials and exposed poultry abattoir workers: a veterinary public health perspective.	J. Oguttu, Dept. Agriculture, Animal Health & Human Ecology, College of Agriculture & Environmental Sciences, Univeristy of South Africa, Pretoria, South Africa; P. Thompson, Dept. of Production Animal Studies, Faculty of Veterinay Science, Univeristy of South Africa, Pretoria, South Africa; J. Picard, Dept. Veterinary Tropical Diseases, Faculty of Veterinary Science, University Pretoria, Pretoria, South Africa; C. Veary, Dept. Paraclinical Sciences, Faculty of Veterinary Science, University Pretoria, Pretoria, South Africa. <i>Email: joaguttu@unisa.ac.za.</i>
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
Tuesday, Dec. 7, 2010 - Salon E - 5th Floor

Section Leader: Yvette Johnson

Time	No.	Title	Authors
8:00 Tues.	096	Reporting of methodological features in observational studies of pre-harvest food safety.	J. Sargeant, D. Kelton, K. Snedeker, L. Wisener, E. Leonard, A. Guthrie, M. Faires, Ontario Veterinary College, Guelph, ON, Canada; A. O'Connor, Iowa State University, Ames, IA; D. Renter, Kansas State University, Manhattan, KS, <i>Email: sargeanj@uoguelph.ca</i> .
8:15	097	Using functional metagenomics to predict the emergence of antibiotic resistance.	R. Singer, K. Lang, J. Anderson, University of Minnesota, St. Paul, MN; J. Handelsman, Yale University, New Haven, CT; S. Schwarz, Institute of Farm Animal Genetics, Friedrich-Loeffler-Institut (FLI), Neustadt-Mariensee, Germany, <i>Email: singe024@umn.edu</i> .
8:30	098	Genotypic and phenotypic evidence for L-fucose utilization by <i>Campylobacter jejuni</i> .	W. Muraoka, Q. Zhang, Iowa State University, Ames, IA, <i>Email: wmuraoka@iastate.edu</i> .
8:45	099	Salicylate functions as efflux pump inducer and promotes the emergence of fluoroquinolone-resistant mutants in <i>Campylobacter</i>	Z. Shen, X. Pu, Q. Zhang, Iowa State University, Ames, IA, <i>Email: szq@iastate.edu</i> .
9:00	100	Molecular characterization of vancomycin-resistant and vancomycin-susceptible <i>Enterococcus faecium</i> isolated from a semi-closed and integrated agri-food system.	R. AMACHAWADI, H. Scott, J. Vinasco, T. Nagaraja, Kansas State University, Manhattan, KS; R. Harvey, T. Poole, Southern Plains Agricultural Research Center, USDA, College Station, TX, <i>Email: agraghav@vet.ksu.edu</i> .
9:15 Tues.	101	Survey of bovine enteric viruses and water quality in the midwestern pasture streams during 2007 to 2009 grazing seasons.	Y. Cho, S. Ensley, K. Yoon, Department of Veterinary Diagnostic and Production Animal Medicine, College of Veterinary Medicine, Iowa State University, Ames, IA; D. Bear, J. Russell, Department of Animal Science, College of Agriculture, Iowa State University, Ames, IA; W. Kim, College of Veterinary Medicine, Chonbuk National University, Jeonju, Korea, Republic of, <i>Email: ycho@iastate.edu</i> .
9:30		Break and Table Top Exhibits – Foyer	
11:45 to	12:30	Business Meeting: Dedication, New Members Introduction, and Graduate Student Competition Awards Presentations	

GASTROENTERIC DISEASES

Monday, Dec. 6, 2010 - Michigan/Michigan State Room - 6th Floor

Section Leaders: David H. Francis and Radhey S. Kaushik

Oral presentations 15 minutes: 10-12 minutes, 3-5 minutes interactive discussion.

Keynote presentations: 35-40 minute oral presentation and a 5-10 minute interactive discussion.

Time	No.	Title	Authors
8:00 Mon.	102	Development of reverse genetics with a full length infectious cDNA of porcine epidemic diarrhea virus	D. Lee, C. Lee, Kyungpook National University, Daegu, Korea, Republic of, <i>Email: gulbay138@knu.ac.kr.</i>
8:15	103	Efficacy of HYPER-EGG K-99 avian egg antibodies on prevention of diarrhea and improvement of survival of colostrum-deprived neonatal calves infected with ETEC K-99 B44.	P. Maiti, S. Cho, Nutratch, Winnipeg, MB, Canada; M. He, Lethbridge Research Center, Lethbridge, AB, Canada; T. McAllister, Lethbridge Research Center, Lethbridge, AB, Canada, <i>Email: dr.pmaiti@nutratchglobal.com.</i>
8:30	104	Inverse relationship between fluid accumulation and adherence of enterotoxigenic <i>Escherichia coli</i> in ligated jejunal loops.	R. Moxley, J. Erume, S. Kachman, University of Nebraska-Lincoln, Lincoln, NE; D. Francis, South Dakota State University, Brookings, SD, <i>Email: rmoxley1@unl.edu.</i>
8:45-9:30 Keynote	105	Unraveling the mysteries of O-antigens in <i>Escherichia coli</i> .	C. DebRoy*, ¹ E. coli Reference Center, Department of Veterinary and Biomedical Sciences, The Pennsylvania State University, University Park, PA 16802.
9:30		Break and Table Top Exhibits – Foyer	
10:00	106	Contribution of PhoA(cj) to Twin Arginine Translocation mediated <i>Campylobacter jejuni</i> function and resilience to Environmental Stresses.	M. Drozd, D. Gangaiah, Z. Liu, G. Rajashekara, The Ohio State University, Wooster, OH, <i>Email: drozd.6@osu.edu.</i>
10:15	107	Epidemiological implications of the invasion associated marker in <i>Campylobacter jejuni</i> isolated from cattle	Y. Sanad, I. Kassem, J. LeJeune, G. Rajashekara, The Ohio State University, Wooster, OH; J. Lin, The University of Tennessee, Knoxville, TN, <i>Email: sanad.1@osu.edu.</i>
10:30	108	An alternative protocol for cultivation of <i>Lawsonia intracellularis</i> .	F. Vannucci, S. Wattanaphansak, C. Gebhart, University of Minnesota, St. Paul, MN, <i>Email: vannu008@umn.edu.</i>
10:45	109	Multilocus sequencing typing system for <i>Lawsonia intracellularis</i> .	M. Kelley, C. Gebhart, S. Oliveira, University of Minnesota, Saint Paul, MN, <i>Email: free0239@umn.edu.</i>
11:00	110	A rabbit infection model for equine proliferative enteropathy.	F. Sampieri, A. Antonopoulos, K. Ball, P. Dowling, D. Hamilton, Dept. of Veterinary Biomedical Sciences, Western College of Veterinary Medicine, University of Saskatchewan, Saskatoon, SK, Canada; C. Gebhart, F. Vannucci, Dept. of Veterinary and Biomedical Sciences, College of Veterinary Medicine, University of Minnesota, St. Paul, MN; A. Allen, Dept. of Veterinary Pathology, Western College of Veterinary Medicine, University of Saskatchewan, Saskatoon, SK, Canada; N. Pusterla, Dept. of Medicine and Epidemiology, School of Veterinary Medicine, University of California at Davis, Davis, CA, <i>Email: fsampierivet@hotmail.com.</i>

GASTROENTERIC DISEASES

Monday, Dec. 6, 2010 - Michigan/Michigan State Room - 6th Floor

Section Leaders: David H. Francis and Radhey S. Kaushik

Oral presentations 15 minutes: 10-12 minutes, 3-5 minutes interactive discussion.

Time	No.	Title	Authors
11:15 Mon.	111	Functional cloning of novel antibiotic resistance genes in chicken gut microflora.	W. Zhou, A. Hunkapiller, J. Lin, University of Tennessee Knoxville, Knoxville, TN, <i>Email: wzhou2@utk.edu</i> .
11:30		Lunch and Table Top Exhibits - Foyer	
1:30	112	Bacterial profiling of enteric microbiota of reticulated giraffes (<i>Giraffa camelopardis reticulata</i>).	A. Johnson, C. Nakatsu, Purdue University, West Lafayette, IN; J. Proudfoot, Indianapolis Zoo, Indianapolis, IN, <i>Email: johns274@purdue.edu</i> .
1:45	113	Survival of <i>Mycobacterium avium</i> subsp. paratuberculosis in bovine monocyte-derived macrophages.	D. McVey, J. Kuszak, R. Barletta, University of Nebraska, Lincoln, NE; C. Chitko-McKown, USDA, ARS, Clay Center, NE, <i>Email: dmcvey2@unl.edu</i> .
2:00	114	Effect of adjuvants on recombinant coccidia antigen vaccination.	R. Parker, SEPPIC Inc, Fairfield, NJ; S. Deville, SEPPIC Inc, Puteaux, France, France; L. Dupuis, SEPPIC Inc, Puteaux,, France; F. Bertrand, SEPPIC Inc, Puteaux, France; E. Lillehoj, S. Lee, K. Lee, M. Park, S. Jang, H. Lillehoj, Animal Parasitic Diseases Laboratory, Animal and Natural Resources Institute, Agricultural Research Service, U.S. Department of Agriculture, Beltsville, MD, <i>Email: robert.parker@airliquide.com</i> .
2:15	115	Lecithinase production by <i>Clostridium perfringens</i> HBS and LDA isolates utilizing drop-plate method of enumeration in conjunction with microplate and agar egg yolk assays.	R. Magnuson, J. Triantis, D. Van Metre, P. Morley, M. Salman, Colorado State University, Fort Collins, CO, <i>Email: roberta.magnuson@colostate.edu</i> .
2:30 Mon.	116	Hypervirulent <i>Clostridium difficile</i> in pigs its public health significance.	P. Fry, P. Pancholi, M. Abley, W. Gebreyes, Ohio State University, Columbus, OH; M. Marcon, Nationwide Children's Hospital, Columbus, OH; S. Thakur, NC State University, Raleigh, NC, <i>Email: fry.175@buckeyemail.osu.edu</i> .
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

Monday, Dec. 6, 2010 - Salons F/G/H - 5th Floor

Section Leader: Isis Mullarky

Oral presentations 15 minutes: 10-12 minutes, 3-5 minutes interactive discussion.

Keynote presentations: 35-40 minute oral presentation and a 5-10 minute interactive discussion.

Time	No.	Title	Authors
		Presiders: Gina Pighetti and Isis K. Mullarky	
8:00 Mon.	117	Regulation of antigen specific T-cell responses by paracrine vitamin D signaling in peripheral blood mononuclear cell cultures.	C. Nelson, D. Beitz, Iowa State University, Ames, IA; B. Nonnecke, T. Reinhardt, R. Waters, J. Lippolis, National Animal Disease Center, ARS, USDA, Ames, IA, <i>Email: cdnelson3@wisc.edu</i> .
8:15	118	Preliminary investigation of B cell immune responses to rotavirus in vitamin A deficient piglets.	K. Chattha, A. Vlasova, C. Siegismund, N. Chen, L. Saif, OARDC, The Ohio State University, Wooster, OH, Email: chattha.2@osu.edu.
8:30	119	Analysis of Bovine Viral Diarrhea Viruses-infected monocytes: Identification of cytopathic and non-cytopathic biotype differences	M. Ammari, F. McCarthy, B. Nanduri, L. Pinchuk, Mississippi State University, Starkville, MS; G. Pinchuk, Mississippi University for Women, Columbus, MS, <i>Email: ammari@cvm.msstate.edu</i> .
8:45	120	Bovine macrophages cleave extracellular traps produced by macrophages and neutrophils in response to Mannheimia Haemolytica and its leukotoxin.	N. Aulik, K. Hellenbrand, C. Czuprynski, University of Wisconsin Madison, Madison, WI, <i>Email: naaulik@wisc.edu</i> .
9:00	121	<i>Histophilus somnii</i> causes Neutrophil Extracellular Trap (NET) formation in bovine neutrophils.	K. Hellenbrand, N. Aulik, J. Rivera, C. Czuprynski, Department of Pathobiological Sciences, School of Veterinary Medicine, University of Wisconsin-Madison, Madison, WI, <i>Email: kmhellenbran@wisc.edu</i> .
9:15	122	Up-regulation of inflammatory mediators and pro-apoptotic genes during Mycoplasma gallisepticum infection.	S. Majumder, J. Mohammed, D. Rood, S. Szczepanek, S. Geary, S. Frasca, L. Silbart, University of Connecticut, Storrs, CT, <i>Email: sanjukta.majumder@uconn.edu</i> .
9:30		Break and Table Top Exhibits – Foyer	
		Presiders: Jamie L. Boehmer and Isis K. Mullarky	
10:00 Mon.	123	Use of dermal fibroblasts to evaluate developmental, and between-animal variation in innate immune response.	B. Green, S. Kandasamy, D. Kerr, University of Vermont, Burlington, VT, <i>Email: bbgreen@uvm.edu</i> .
10:15	124	Response of cultured fibroblasts to LPS reflects in vivo response to E. coli mastitis.	S. Kandasamy, B. Green, D. Kerr, University of Vermont, Burlington, VT, <i>Email: skandasa@uvm.edu</i> .
10:30	125	Distribution of polysulfated proteoglycans in the equine digital lamellae: implications for the pathogenesis of laminitis.	E. Pawlak, L. Wang, D. Alfandari, S. Black, University of Massachusetts Amherst, Amherst, MA; P. Johnson, College of Veterinary Medicine, University of Missouri Columbi, Columbia, MO; J. Belknap, School of Veterinary Medicine, The Ohio State University, Columbus, OH, <i>Email: epawlak@vasci.umass.edu</i> .
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IMMUNOLOGY

Monday, Dec. 6, 2010 - Salons F/G/H - 5th Floor

Section Leader: Isis Mullarky

Time	No.	Title	Authors
10:45	126	Depression of gene expression and elevated ADAMTS-4 degradation of versican in equine laminitis	L. Wang, E. Pawlak, D. Alfandari, S. Black, Department of Veterinary and Animal Science, University of Massachusetts, Amherst, MA; P. Johnson, Department of Veterinary Medicine and Surgery, College of Veterinary Medicine, University of Missouri, Columbia, MO; J. Belknap, School of Veterinary Medicine, The Ohio State University, Columbus, OH, <i>Email: lew@vasci.umass.edu</i> .
11:00	127	Environment affects interferon-gamma (IFN- γ) production in neonatal foals through possible effects on dendritic cells.	L. Sun, A. Betancourt, A. Page, E. Oberst, A. Adams, N. Combs, D. Horohov, Gluck Equine Research Center, Lexington, KY, <i>Email: lsun4@uky.edu</i> .
11:15			
11:30		Lunch and Table Top Exhibits - Foyer	
		Presiders: Doug Bannerman and Lorraine M. Sordillo	
1:30-2:15 Keynote	128	Interplay of antimicrobial peptides and interferons in Porcine Reproductive and Respiratory Syndrome Virus infections.	F. Blecha, Kansas State University, Manhattan, KS, <i>Email: blecha@vet.k-state.edu</i> .
2:15 Mon.	129	Mucosal immunization with biodegradable PLG- nanoparticles elicits effective anti-PRRSV immune responses in Pigs	V. Dwivedi, C. Manickam, B. Binjawadagi, R. Patterson, K. Dodson, R. Gourapura, Food Animal Health Research program, OARDC, The Ohio State University, Wooster, OH, <i>Email: dwivedi.6@osu.edu</i> .
2:30	130	Replication competent recombinant PRRS viruses expressing indicator proteins and antiviral cytokines.	Y. Sang, R. Rowland, F. Blecha, Kansas State University, Manhattan, KS, <i>Email: ysang@vet.k-state.edu</i> .
2:45		Break and Table Top Exhibits – Foyer	
		Presiders: Amanda A. Adams and David Kerr	
3:00	131	Induction of robust influenza A-specific IFN- γ responses in pigs using alphavirus-derived replicon particles.	B. Russell, R. Vander Veen, M. Mogler, D. Harris, Iowa State University, Ames, IA, <i>Email: candoia@iastate.edu</i> .
3:15	132	The expression of potentially protective dual oxidase enzymes (Duox1 And Duox2) in the female bovine reproductive tract.	B. Adu-Addai, C. Mackenzie, D. Agnew, Michigan State University, East Lansing, MI; A. Langerveld, Genemarkers, LLC, Portage, MI, <i>Email: aduaddai@msu.edu</i> .
3:30	133	Mucosal adjuvanticity of <i>M.tb</i> whole cell lysate to PRRSV live vaccine leads to a better immunostimulation and protection against PRRSV challenge.	C. Manickam, V. Dwivedi, M. Khatri, J. Miller, R. Patterson, R. Gourapura, FAHRP, OARDC, The Ohio State University, wooster, OH; T. Papenfuss, Department of Veterinary Biosciences, The Ohio State University, wooster, OH, <i>Email: manickam.4@osu.edu</i> .

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IMMUNOLOGY
Monday, Dec. 6, 2010 - Salons F/G/H - 5th Floor
Section Leader: Isis Mullarky

Time	No.	Title	Authors
3:45 Mon.	134	Efficacy of canine influenza virus (H3N8) vaccine in dogs co-infected with CIV and <i>Streptococcus equi zooepidemicus</i> .	R. Schultz, L. Larson, J. Henningson, P. Sharp, B. Thiel, University of Wisconsin-Madison, Madison, WI; M. Deshpande, T. Davis, T. Wasmoen, N. Lakshmanan, Intervet Schering Plough Animal Health, Elkhorn, NE, <i>Email: rschultz@svm.vetmed.wisc.edu</i> .
4:00	135	Differential expression of the porcine heavy chain immunoglobulin repertoire following Porcine Reproductive and Respiratory Syndrome Virus (PRRSV) infection.	J. Schwartz, J. Abrahante, M. Murtaugh, University of Minnesota, St. Paul, MN, <i>Email: schwa753@umn.edu</i> .
4:15	136	A time course for increased susceptibility to <i>Staphylococcus aureus</i> respiratory infection post-influenza in a swine model.	E. Smith, J. Deventhiran, S. Kumar, S. Elankumaran, I. Mullarky, Virginia Polytechnic Institute and State University, Blacksburg, VA, <i>Email: allivt08@vt.edu</i> .
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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IMMUNOLOGY

Tuesday, Dec. 7, 2010 - Salons F/G/H - 5th Floor

Section Leader: Isis Mullarky

Time	No.	Title	Authors
		Presiders: Amanda A. Adams and Isis K. Mullarky	
8:30 Tues.	137	Brucella outer membrane vesicles-polaxamer mixture as a vaccine for <i>B. melitensis</i> in a mouse model.	N. Jain, S. Boyle, N. Sriranganathan, Virginia Tech, Blacksburg, VA; A. Rodriguez, E. Avila-Calderon, A. López-Merino, Instituto Politécnico Nacional, Mexico, Mexico, <i>Email: njain80@vt.edu.</i>
8:45	138	FMDV serotype O ₁ peptide-based vaccines incorporating xeno-epitopes refocus humoral immune responses away from a major decoy epitope to an alternative immunogenic site.	R. Barrette, Animal and Plant Health Inspection Services, United States Department of Agriculture, Greenport, NY; S. Szczepanek, D. Rood, L. Silbart, Center of Excellence for Vaccine Research, University of Connecticut, Storrs, CT, <i>Email: steven.szczepanek@uconn.edu.</i>
9:00	139	Epitope mapping of the E2 glycoprotein of Classical Swine Fever Virus	A. Kozlov, L. Kostina, A. Zaberezhny, T. Aliper, E. Nepoklonov, D.I.Ivanovski Virology Institute, NARVAC R&D, Moscow, Russian Federation, <i>Email: zaberezhny@narvac.com.</i>
9:15 Tues.	140	G-CSF analogue treatment increases peripheral neutrophil numbers in pigs - a potential alternative for in-feed antibiotics.	C. Loving, S. Brockmeier, D. Bayles, J. Greenlee, K. Lager, M. Kehrli, USDA-ARS-National Animal Disease Center, Ames, IA, <i>Email: crystal.loving@ars.usda.gov.</i>
9:30		Break and Table Top Exhibits – Foyer	
		Presiders: Paul Coussens and Isis K. Mullarky	
10:00	141	Temporal genes expression induced by MDV in Marek's disease-resistant and -susceptible inbred chickens	J. Luo, A. Mitra, Y. Yu, F. Tian, P. Yuan, J. Song, University of Maryland, College Park, MD; H. Zhang, Avian Disease and Oncology Laboratory, East Lansing, MI; H. Zhou, Texas A&M University, College Station, TX, <i>Email: jluo1@umd.edu.</i>
10:15	142	Effect of serum composition on the functionality of bovine peripheral blood mononuclear cells.	C. Ster, P. Lacasse, Agriculture and Agri Food Canada, Sherbrooke, QC, Canada; M. Loiselle, Université de Sherbrooke, Sherbrooke, QC, Canada, <i>Email: celine.ster@usherbrooke.ca.</i>
10:30	143	TLR dependent dendritic cell activation and clearance of <i>Brucella abortus</i> vaccine and pathogenic strains <i>in vivo</i> and <i>in vitro</i> .	N. Surendran, University of Maryland School of Medicine, Baltimore, MD; E. Hiltbold-Schwartz, Wake Forest, Winston-Salem, NC; N. Sriranganathan, B. Heid, S. Boyle, M. Makris, K. Zimmerman, S. Witonsky, Virginia Maryland Regional College of Veterinary Medicine, Blacksburg, VA, <i>Email: switonsk@vt.edu.</i>
10:45 Tues.	144	Immune responses of equine respiratory epithelial cells to equine herpesvirus-1.	A. Quintana, G. Landolt, K. Annis, G. Soboll Hussey, Colorado State University, Fort Collins, CO, <i>Email: husseygs@colostate.edu.</i>
11:45 to	12:30	Business Meeting: Dedication, New Members Introduction, and Student Competition Awards	

RESPIRATORY DISEASES

Monday, Dec. 6, 2010 - Indiana/Iowa Room 6th Floor

Section Leaders: Amelia Woolums and Christopher Chase

Oral presentations 15 minutes: 10-12 minutes, 3-5 minutes interactive discussion.

Keynote presentations: 35-40 minute oral presentation and a 5-10 minute interactive discussion.

Time	No.	Title	Authors
8:00 Mon.	145	Porcine Reproductive And Respiratory Syndrome Virus (PRRSV) in serum and oral fluid samples from individual boars: Will oral fluid replace serum for PRRSV surveillance?	A. Kittawornrat, W. Chittick, J. Johnson, J. Prickett, C. Wang, T. Schwartz, K. Schwartz, D. Whitney, C. Olsen, J. Zimmerman, Iowa State University, Ames, IA; M. Engle, PIC North America, Hendersonville, TN; D. Patnayak, University of Minnesota, St Paul, MN, <i>Email: apisitk@iastate.edu</i> .
8:15	146	Detection of Anti-PRRSV antibodies in oral fluid samples from individual boars using a commercial PRRS ELISA.	A. Kittawornrat, J. Prickett, J. Johnson, D. Whitney, C. Olsen, T. Schwartz, K. Schwartz, C. Wang, J. Zimmerman, Iowa State University, Ames, IA; M. Engle, PIC North America, Hendersonville, TN, <i>Email: apisitk@iastate.edu</i> .
8:30	147	Development of a new immunochromatographic strip assay to detect the infection caused by PRRSV in pigs.	M. Achacha, A. Kheyar, A. Bensari, AriVac Inc, Saint -Hyacinthe, QC, Canada, <i>Email: achacha@sympatico.ca</i> .
8:45	148	Influenza antibody detection in experimentally inoculated swine over time using a commercially available nucleoprotein ELISA.	C. Irwin, J. Prickett, A. Kittawornrat, J. Johnson, C. Wang, J. Zimmerman, Iowa State University College of Veterinary Medicine, Ames, IA, <i>Email: cirwin@iastate.edu</i> .
9:00	149	A predictive model for the detection of influenza antibodies over time using a commercially available nucleoprotein ELISA.	C. Irwin, J. Prickett, A. Kittawornrat, J. Johnson, C. Wang, J. Zimmerman, Iowa State University College of Veterinary Medicine, Ames, IA, <i>Email: cirwin@iastate.edu</i> .
9:15	150	Influenza antibody detection in experimentally inoculated swine over time using two commercially-available indirect ELISAs.	C. Irwin, J. Prickett, A. Kittawornrat, J. Johnson, C. Wang, J. Zimmerman, Iowa State University College of Veterinary Medicine, Ames, IA, <i>Email: cirwin@iastate.edu</i> .
9:30		Break and Table Top Exhibits – Foyer	
9:45	151	Novel SYBR real-time PCR assay for detection and differentiation of Mycoplasma species in biological samples from various hosts.	J. Trujillo, P. Nara, Center for Advanced Host Defenses, Immunobiotics and Translational Comparative Medicine, College of Veterinary Medicine, Iowa State University, Ames, IA; E. Strait, Veterinary Diagnostic Laboratory, Iowa State University, Ames Iowa, Ames, IA, <i>Email: itruiill@iastate.edu</i> .
10:00 Mon.	152	Genotypic characterization and quantification of <i>Mycoplasma bovis</i> in naturally occurring respiratory disease in feedlot cattle.	J. Caswell, F. Castillo, K. Bateman, H. Cai, M. Clark, L. Parker, P. McRaid, R. Travis, University of Guelph, Guelph, ON, Canada; M. Archambault, University of Montreal, St. Hyacinthe, QC, Canada, <i>Email: icaswell@uoguelph.ca</i> .
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RESPIRATORY DISEASES

Monday, Dec. 6, 2010 - Indiana/Iowa Room 6th Floor

Section Leaders: Amelia Woolums and Christopher Chase

Time	No.	Title	Authors
10:15 Mon.	153	Bacterial isolations from lungs of beef calves with bronchopneumonia associated with acute bovine respiratory disease	D. McVey, J. Kuszak, University of Nebraska, Lincoln, NE, <i>Email: dmcvey2@unl.edu</i> .
10:30	154	Bovine tuberculosis at livestock-human interface in the Pastoralist communities of Southern Ethiopia.	B. Gumi, E. Schelling, J. Zinsstag, Swiss Tropical and public health Institute, Basel, Switzerland; A. Aseffa, Armauer Hansen Research Institute, Addis Ababa, Ethiopia; D. Young, Department of Infectious Disease and Microbiology, Imperial College, London, United Kingdom, <i>Email: balako.gumi-donde@stud.unibas.ch</i> .
10:45	155	Comparative efficacy and immunogenicity of RSV and BRSV vaccines in experimental Bovine Respiratory Syncytial Virus infection.	L. Gershwin, M. Shao, H. VanHoosear, M. Anderson, University of California, Davis, Davis, CA, <i>Email: ljgershwin@ucdavis.edu</i> .
11:00	156	Impact of route and timing of multivalent respiratory vaccination in the face of maternal antibody (IFOMA) on immune responses to booster vaccination at weaning in beef calves.	L. Berghaus, D. Hurley, R. Berghaus, M. Pence, R. Ellis, J. Saliki, K. Hurley, K. Galland, A. Woolums, University of Georgia, Athens, GA; B. Burdett, Intervet/Schering-Plough Animal Health, Cairo, NE; S. Nordstrom, Intervet/Schering Plough Animal Health, Middlebrook, VA, <i>Email: ljberg@uga.edu</i> .
11:15	157	Evaluation of skin samples by RT-PCR following immunization with a modified-live Bovine Viral Diarrhea Virus vaccine.	E. Corbett, D. Grooms, Dept. of Large Animal Clinical Sciences, College of Veterinary Medicine, Michigan State University, East Lansing, MI; S. Bolin, Diagnostic Center for Population and Animal Health, College of Veterinary Medicine, Michigan State University State University, East Lansing, MI, <i>Email: corbett2@cvm.msu.edu</i> .
11:30		Lunch and Table Top Exhibits - Foyer	
1:30 Mon.	158	Establishment of a DNA-launched infection cDNA clone for a highly-pathogenic strain of type II PRRSV (strain VR2385): Identification of a naturally-occurring deletion in the nsp2 region that enhances virus replication.	Y. Ni, Y. Huang, X. Meng, Virginia Polytechnic Institute and State University, Blacksburg, VA; T. Opriessnig, Iowa State University, Ames, IA, <i>Email: nyy7@vt.edu</i> .
1:45	159	Modulation of host innate immune response by the PRRSV nucleocapsid protein in porcine alveolar macrophages	M. Sagong, c. Lee, Kyungpook National University, Daegu, Korea, Republic of, <i>Email: fiog40@naver.com</i> .
2:00	160	Enhanced activation of swine dendritic cells by NS1-truncated swine influenza viruses.	M. Khatri, Food Animal Health Research Program, Ohio Agricultural Research and Development Center, The Ohio State University, Wooster, OH; D. Franklin, C. Lee, Food Animal Health Research Program, Ohio Agricultural Research and Development Center, The Ohio State University, Wooster, OH, <i>Email: khatri.14@osu.edu</i> .

RESPIRATORY DISEASES

Monday, Dec. 6, 2010 - Indiana/Iowa Room 6th Floor

Section Leaders: Amelia Woolums and Christopher Chase

Time	No.	Title	Authors
2:15 Mon.	161	Enhanced pneumonia with pandemic 2009 A/H1N1 swine influenza virus in pigs vaccinated with an inactivated δ -cluster H1N2 vaccine.	P. Gauger, A. Vincent, K. Lager, C. Loving, National Animal Disease Center, Ames, IA; B. Janke, Iowa State University, Ames, IA, <i>Email: pcgauger@iastate.edu</i> .
2:30	162	Evaluation of the Newcastle disease virus F and HN proteins in protective immunity using a recombinant avian paramyxovirus type-3 vector in chickens.	S. Kumar, B. Nayak, S. Samal, University of Maryland, College Park, MD; P. Collins, National Institute of Health, Bethesda, MD, <i>Email: sachin22@umd.edu</i> .
2:45		Break and Table Top Exhibits – Foyer	
3:00	163	Cross-protection of pigs vaccinated and challenged with delta-1 and delta-2 sub-cluster H1 Swine Influenza Viruses (SIV).	V. Rapp-Gabrielson, G. Nitzel, J. Czach, T. Hildebrand, S. Behan, L. Taylor, Pfizer Animal Health, Kalamazoo, MI; E. Wicklund, Pfizer Global Manufacturing, Lincoln, NE, <i>Email: vicki.i.rapp-gabrielson@pfizer.com</i> .
3:15	164	The influence of fetal PCV2 infection on lifetime performance and vaccine efficacy.	J. Lowe, University of Illinois, Urbana, IL; L. Greiner, Innovative Swine Solutions, Carthage, IL, <i>Email: jlowe@illinois.edu</i> .
3:30 Mon.	165	Innate immune responses are enhanced in pigs after sequential infection with influenza virus and <i>Haemophilus parasuis</i> .	C. Loving, S. Brockmeier, A. Vincent, USDA-ARS-National Animal Disease Center, Ames, IA, <i>Email: crystal.loving@ars.usda.gov</i> .
3:45-4:30	166	Polymicrobial respiratory disease in pigs	T. Opriessnig, Iowa State University, Ames, IA, <i>Email: tanjaopr@iastate.edu</i> .
Keynote			
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	

VECTOR-BORNE AND PARASITIC DISEASES
Monday, Dec. 6, 2010 - Denver/Houston - 5th Floor
Section Leader: Roman Ganta

Oral presentations 15 minutes: 10-12 minutes, 3-5 minutes interactive discussion.

Keynote presentations: 35-40 minute oral presentation and a 5-10 minute interactive discussion.

Time	No.	Title	Authors
8:00 Mon.	167	A fluorescent bead-based multiplex assay for the simultaneous detection of antibodies to <i>B. burdorferi</i> outer surface protein A (OspA), OspC and OspF in canine serum.	B. Wagner, H. Freer, A. Rollins, H. Erb, Cornell University, Ithaca, NY; P. Meeus, Pfizer Animal Health, Kalamazoo, MI, <i>Email: bw73@cornell.edu</i> .
8:15	168	Functional analysis of genes differentially expressed in tick cells in response to <i>Anaplasma phagocytophilum</i> infection.	E. Blouin, A. Busby, K. Kocan, J. Fuente, Oklahoma State University, Stillwater, OK, <i>Email: edmour.blouin@okstate.edu</i> .
8:30	169	Survey of <i>Ehrlichia</i> and <i>Anaplasma</i> species in white tailed deer by real-time RT-PCR or PCR and DNA sequence analysis.	C. Katragadda, C. Cheng, L. Fox, M. Dryden, R. Ganta, Kansas State University, Manhattan, KS, <i>Email: chakri@ksu.edu</i> .
8:45	170	Preliminary development of an approach to rapidly identify and isolate <i>Ehrlichia chaffeensis</i> from naturally infected domestic hosts.	S. YOO-EAM, R. Stoffel, K. Curtis, M. Whitney, A. Bermudez, R. Stich, University of Missouri, Columbia, MO; P. Rajala-Schulz, Ohio State University, Columbus, OH, <i>Email: sy262@mail.missouri.edu</i> .
9:00	171	Utilization of a peptide based enzyme linked immunosorbant assay for the detection of bovine anti- <i>E. chaffeensis</i> antibodies.	R. Stoffel, A. Boyles, R. Stich, University of Missouri, Columbia, MO, <i>Email: stoffelrt@missouri.edu</i> .
9:15	172	The <i>Index Catalogue of Medical and Veterinary Zoology</i> , the quintessential parasitology literature research tool, now digitized, preserved, and freely available worldwide.	S. Ewing, H. Moberly, Oklahoma State University, Stillwater, OK; E. Carrigan, Texas A&M University, College Station, TX, <i>Email: sidney.ewing@okstate.edu</i> .
9:30		Break and Table Top Exhibits – Foyer	
10:00-10:45 Keynote	173	Bovine Anaplasmosis: An Overview of current challenges.	K. Kocan, J. de la Fuente, E. Blouin, D. Step, Oklahoma State University, Stillwater, OK; J. Coetzee, Kansas State University, Manhattan, KS, <i>Email: Katherine.Kocan@okstate.edu</i> .
10:45	174	Detection of <i>E. chaffeensis</i> in post-mortem blood and tissue samples from an elk using real-time PCR.	R. Stoffel, G. Johnson, R. Stich, University of Missouri, Columbia, MO, <i>Email: stoffelrt@missouri.edu</i> .
11:00	175	The effect of anthelmintics on proinflammatory cytokine responses in treated horses.	A. Betancourt, E. Lyons, D. Horohov, Gluck Equine Research Center, Lexington, KY, <i>Email: abeta2@uky.edu</i> .
11:15	176	Purification of recombinant anaplasma appendage associated protein for studies that involve understanding its biological activity.	B. Dhagat-Mehta, R. Stich, University of Missouri, Columbia, MO, <i>Email: dhagatb@missouri.edu</i> .
11:30		Lunch 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	

VIRAL PATHOGENESIS

Monday, Dec. 6, 2010 - Los Angeles/Miami/Scottsdale Rooms - 5th Floor

Section Leader: Kyoung-Jin Yoon

Oral presentations 15 minutes: 10-12 minutes, 3-5 minutes interactive discussion.

Keynote presentations: 35-40 minute oral presentation and a 5-10 minute interactive discussion.

Time	No.	Title	Authors
8:00 Mon.	177	Replacement of the replication factors of Porcine Circovirus (PCV) Type 2 with those of PCV Type 1 greatly enhances viral replication <i>in vitro</i> .	N. Beach, N. Juhan, L. Cordoba, X. Meng, Virginia Tech, Blacksburg, VA, <i>Email: beachnm@gmail.com</i> .
8:15	178	A chimeric Porcine circovirus (PCV) with the capsid gene of PCV2b cloned in the genomic backbone of PCV1 is attenuated <i>in vivo</i> and protects pigs against PCV2a and PCV2b challenge.	N. Beach, S. Ramamoorthy, X. Meng, Virginia Tech, Blacksburg, VA; T. Opriessnig, Iowa State University, Ames, IA; S. Wu, Fort Dodge Animal Health, Inc, Fort Dodge, IA, <i>Email: beachnm@gmail.com</i> .
8:30	179	Transmission of PCV2 from sow to piglet in the farrowing room.	C. Dvorak, M. Lilla, M. Murtaugh, University of Minnesota, St.Paul, MN, <i>Email: dvora013@umn.edu</i> .
8:45	180	Diversity of porcine torque teno viruses.	J. Bai, B. An, R. Hesse, G. Anderson, Kansas State University, Manhattan, KS, <i>Email: jbai@vet.ksu.edu</i> .
9:00	181	Development of SYBR green-based real-time PCR assays for quantification and differential detection of species-specific porcine Torque teno viruses (TTV)	Y. Huang, B. Dryman, K. Harrall, X. Meng, Virginia Tech, Blacksburg, VA; E. Vaughn, M. Roof, Boehringer Ingelheim Vetmedica, Inc., Ames, IA, <i>Email: yhuang@vt.edu</i> .
9:15	182	Serodiagnosis of porcine Torque teno virus (TTV) infection in pigs by Western blot and indirect ELISAs	Y. Huang, K. Harrall, B. Dryman, X. Meng, Virginia Tech, Blacksburg, VA; T. Opriessnig, Iowa State University, Ames, IA; E. Vaughn, M. Roof, Boehringer Ingelheim Vetmedica, Inc., Ames, IA, <i>Email: yhuang@vt.edu</i> .
9:30		Break and Table Top Exhibits – Foyer	
10:00 Mon.	183	Interaction between structural Core protein of Classical Swine Fever Virus with IQGAP protein appears as essential for virus virulence in swine	D. Gladue, L. Holinka, I. Fernandez-Sainz, M. Prarat, M. Borca, Plum Island Animal Disease Center, ARS, USDA, Greenport, NY; Z. Lu, Plum Island Animal Disease Center, DHS, Greenport, NY; G. Risatti, Department of Pathobiology, University of Connecticut, Storrs, CT, <i>Email: manuel.borca@ars.usda.gov</i> .
10:15	184	The effect of glycosylation on antigenicity and immunogenicity of Classical Swine Fever Virus envelope proteins.	B. Gavrilov, K. Rogers, G. Risatti, Department of Pathobiology, University of Connecticut, Storrs, CT; I. Fernandez-Sainz, L. Holinka, M. Borca, Plum Island Animal Disease Center, ARS, USDA, Greenport, NY, <i>Email: guillermo.risatti@uconn.edu</i> .
10:30	185	Bovine viral diarrhea virus fetal infection following immunization with a contaminated modified-live virus vaccine.	R. Palomares, Auburn University, College of Veterinary Medicine, Department of Clinical Sciences, Auburn, AL; P. Walz, D. Givens, Auburn University, College of Veterinary Medicine, Department of Pathobiology, Department of Clinical Sciences, Auburn, AL; M. Marley, H. Walz, K. Brock, Auburn University, Department of Pathobiology, Auburn, AL; M. Caldwell, Auburn University, Department of Pathobiology, Department of Clinical Sciences, Auburn, AL, <i>Email: palomnr@auburn.edu</i> .

VIRAL PATHOGENESIS

Monday, Dec. 6, 2010 - Los Angeles/Miami/Scottsdale Rooms - 5th Floor

Section Leader: Kyoung-Jin Yoon

Time	No.	Title	Authors
10:45	186	A Bovine Viral Diarrhea Virus type 1b subunit vaccine induces neutralizing antibodies in calves.	K. Sirigireddy, R. Bey, B. Hause, D. Stine, R. Simonson, Newport Laboratories, Worthington, MN, <i>Email: ksirigireddy@newportlabs.com</i> .
11:00	187	Bovine viral diarrhea virus N ^{pro} antagonism of IFN-I mediates enhancement of bovine respiratory syncytial virus replication during co-infections.	A. Alkheraif, C. Topliff, C. Kelling, School of Veterinary Medicine and Biomedical Sciences, University of Nebraska, Lincoln, NE; R. Donis, Influenza Division, Center for Disease Control and Prevention, Atlanta, GA; G. Meyers, Department of Clinical Virology, Federal Research Centre for Virus Diseases of Animals, Tübingen, Germany; K. Eskridge, Department of Statistics, University of Nebraska, Lincoln, NE, <i>Email: ctopliff2@unl.edu</i> .
11:15	188	Antigenic relationships among avian paramyxovirus serotypes	B. Nayak, S. Kumar, F. Dias, A. Paldurai, A. Samuel, S. Xiao, S. Samal, University of Maryland, College Park, MD; P. Collins, Laboratory of Infectious Diseases (LID), NIAID-NIH, Bethesda, MD, <i>Email: bnayak@umd.edu</i> .
11:30		Lunch and Table Top Exhibits - Foyer	
1:30 Mon.	189	Inhibition of NF-kappa B activation and suppression of interferon-beta production by Nsp1 alpha subunit of PRRS virus.	C. Song, D. Yoo, University of Illinois at Urbana-Champaign, Urbana, IL; P. Krell, University of Guelph, Guelph, ON, Canada, <i>Email: chsong@illinois.edu</i> .
1:45	190	Porcine reproductive and respiratory syndrome virus-associated glycans are potential targets for virus neutralization.	J. Li, M. Murtaugh, University of Minnesota, St. Paul, MN; S. Tao, R. Orlando, University of Georgia, Athens, GA, <i>Email: lixxx443@umn.edu</i> .
2:00	191	Broader cross neutralization against PRRS viruses conferred by chimeric viruses containing structural gene fragments from distinct heterologous strains	D. Sun, Y. Cho, K. Yoon, College of Veterinary Medicine, Iowa State University, Ames, IA; W. Kim, College of Veterinary Medicine, Chonbuk National University, Jeonju, Korea, Republic of; S. Cha, E. Choi, National Veterinary Research and Quarantine Service, Anyang, Korea, Republic of, <i>Email: sundong@iastate.edu</i> .
2:15	192	Genetic variation of PRRSV ORF5 following modified live PRRSV vaccination in a PRRSV positive herd.	T. Hoonsuwan, T. Tripipat, P. Kitrunloadjanaporn, S. Laopracha, D. Nilubol, Chulalongkorn University, Bangkok, Thailand, <i>Email: dnilubol@yahoo.com</i> .
2:30	193	Development of Transgenic Mouse Model Susceptible to Porcine Epidemic Diarrhea Virus.	J. Park, H. Shin, Chungnam National University, Daejeon, Korea, Republic of, <i>Email: liebe7867@hanmail.net</i> .
2:45		Break and Table Top Exhibits – Foyer	

(continued)

VIRAL PATHOGENESIS

Monday, Dec. 6, 2010 - Los Angeles/Miami/Scottsdale Rooms - 5th Floor

Section Leader: Kyoung-Jin Yoon

Time	No.	Title	Authors
3:00 Mon.	194	Genetic and antigenic characterization of H1 influenza viruses from United States swine prior to the emergence of the 2009 pandemic H1N1	A. Lorusso, A. Vincent, M. Harland, D. Alt, D. Bayles, K. Lager, National Animal Disease Center, USDA-ARS, Ames, IA; S. Swenson, USDA/APHIS/NVSL, Ames, IA; M. Gramer, University of Minnesota, St. Paul, MN; C. Russell, D. Smith, N. Lewis, University of Cambridge, Cambridge, United Kingdom, <i>Email: alessio.lorusso@ars.usda.gov</i> .
3:15	195	Viral reassortment and transmission after coinfection of pigs with Northern American triple reassortant and Eurasian H1N1 swine influenza viruses.	W. Ma, Q. Liu, J. Ma, J. Richt, Kansas State University, Manhattan, KS; A. Garcia-Sastre, Mount Sinai School of Medicine, New York, NY; R. Webby, St. Jude Children's Research Hospital, Memphis, TN, <i>Email: wma@vet.k-state.edu</i> .
3:30	196	Influenza Infection in Cats.	A. Ali, M. Khatri, C. Lee, Food Animal Health Research Program, Ohio Agricultural Research Development Center, The Ohio State University, Wooster, OH; K. Hayes-Ozello, J. Daniels, L. Mathes, College of Veterinary Medicine, The Ohio State University, Columbus, OH; A. Eladl, Department of Poultry Diseases, Faculty of Veterinary Medicine, Mansoura University, Mansoura, Egypt; Y. Zhang, Animal Disease Diagnostic Laboratory, Ohio Department of Agriculture, Reynoldsburg, OH, <i>Email: ali.178@buckeyemail.osu.edu</i> .
3:45	197	Comparative pathogenicity of novel avian-origin canine influenza virus H3N2 in various species.	B. Kang, H. Moon, M. Yeom, S. Han, D. Son, T. Oh, J. Hwang, J. Kim, D. Song, Green Cross Veterinary Products, Yongin, Korea, Republic of, <i>Email: paransearo@naver.com</i> .
4:00	198	Effective small interfering RNA cocktails targeting viral and avian genes as an alternative to vaccination for avian influenza.	L. Linke, J. Triantis, M. Salman, Colorado State University, Fort Collins, CO, <i>Email: Lyndsey.Linke@colostate.edu</i> .
4:15 Mon.	199	Viral induced inflammatory cytokines, toll like receptors and cytotoxic T cells components in IBDV- infected chickens.	A. Rauf, M. Khatri, M. Murgia, K. Jung, Y. Saif, Food Animal Health Research Program, College of Veterinary Medicine, Ohio Agricultural Research and Development Center, The Ohio State University, Wooster, OH, <i>Email: rauf.4@osu.edu</i> .
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	

(continued)

VIRAL PATHOGENESIS

Tuesday, Dec. 7, 2010 - Los Angeles/Miami/Scottsdale Rooms - 5th Floor

Section Leader: Kyoung-Jin Yoon

Time	No.	Title	Authors
8:00 Tues.	200	Molecular characterization of fowl adenovirus fiber genes.	H. Grgic, E. Nagy, University of Guelph, Ontario Veterinary College, Guelph, ON, Canada; D. Ojkic, University of Guelph, Animal Health Laboratory, Guelph, ON, Canada, <i>Email: hgrgic@uoquelp.ca</i> .
8:15- 9:00 Keynote	201	New and emerging diseases: A very personal perspective.	F. Murphy, University of Texas Medical Branch, Galveston, TX, <i>Email: famurphy@utmb.edu</i> .
9:00	202	Infection of PBMC with Neuropathogenic Equine Herpesvirus Type 1 Ab4 strain induces interferon-alpha and modulates interleukin-10 production.	B. Wagner, C. Wimer, H. Freer, H. Erb, Cornell University, Ithaca, NY; A. Damiani, N. Osterrieder, Freie Universität Berlin, Berlin, Germany, <i>Email: bw73@cornell.edu</i> .
9:15 Tues.	203	Equine herpesvirus type-1 modulates CCL2, CCL3, CCL5, CXCL9, and CXCL10 chemokine expression.	C. Wimer, B. Wagner, Cornell University, College of Veterinary Medicine, Ithaca, NY; A. Damiani, N. Osterrieder, Institut für Virologie, Freie Universität Berlin, Berlin, Germany, <i>Email: clwimer1@yahoo.com</i> .
9:30		Break and Table Top Exhibits – Foyer	
11:45 to	12:30	Business Meeting: Dedication, New Members Introduction, and Graduate Student Competition Awards Presentations	

SYMPOSIUM PROGRAMS

AAVI/ACVM Symposium 2010
Emerging and Re-Emerging Zoonotic Pathogens
Sunday December 5, 2010, 1:30-5 PM
Chicago, Illinois
[prior to the CRWAD meetings]

Co-organizers:

Christopher C. L. Chase, Chairman, ACVM Continuing Education Committee
South Dakota State University, Brookings, SD

Paul Coussens, President-Elect, AAVI
Michigan State University, East Lansing, MI

Speakers and tentative topics (40 min talk; 5 min questions)

1:30-2:00 PM: Dick Bowen, Animal Reproduction and Biotechnology Laboratory (ARBL), Colorado State University, Fort Collins, Colorado
Title Overview - Emerging and Re-Emerging Zoonotic Pathogens.

2:00-2:45 PM: Thomas G. Ksiazek, Galveston National Laboratory, The University of Texas Medical Branch, Galveston, Texas
Title: Rift Valley fever virus, a zoonotic disease of high consequence for both animal and human health.

2:45-3:00 PM: Break

3:00-3:45 PM: Ray Waters, National Animal Disease Center, Ames, Iowa
Title: Bovine tuberculosis: Immune response and vaccine efficacy studies.

3:45-4:30 PM: Juergen Richt, Regents Distinguished Professor and Director, Center of Excellence for Emerging and Zoonotic Animal Diseases (CEEZAD), Kansas State University, College of Veterinary Medicine, Manhattan, KS
Title: The Role of the Pig in Pandemic Flu.

4:30-5:00 PM: Panel Discussion

5:00 PM Adjourn

AAVI-ACVM SYMPOSIUM

1:30 PM

Overview - Emerging and Re-Emerging Zoonotic Pathogens. Richard Bowen, Department of Biomedical Sciences, Animal Reproduction and Biotechnology Laboratory, Colorado State University, Fort Collins, CO.

Recent years have witnessed repeated episodes of high profile infectious diseases that have significantly impacted human health and appear to originate among non-human animals, and there is objective data supporting an increase in such emergence events over the past half-century. The concept of emerging or re-emerging zoonotic disease typically refers to newly recognized or evolved pathogens that induce disease in humans or to situations in which previously known pathogens expand their geographic or host ranges. Prominent examples of such agents include the viruses that cause AIDS, SARS, Nipah, Hendra, Ebola, avian and swine influenza, and a variety of vector-borne syndromes, and bacteria such as enterotoxigenic *E. coli*. Several factors have been identified as drivers of emergence, among which anthropogenic changes to the environment (deforestation, encroachment), social practices (bushmeat trade) and enhanced transportation appear to be particularly important. It seems clear that a large fraction of newly recognized zoonotic pathogens originate from wildlife reservoirs. It is thus understandable that global hot-spots for emergence are centered in regions of high species diversity, and that the bats and rodents, which collectively represent a large fraction of mammalian species, are increasingly recognized as important sources of such pathogens. The critical challenges are to better understand factors promoting emergence, to implement surveillance programs capable of recognizing incipient emergence, and to set in place mechanisms to minimize the impact of major events.

2:00 PM

Rift Valley fever virus, a zoonotic disease of high consequence for both animal and human health. T.G. Ksiazek, DVM, PhD. Galveston National Laboratory, Departments of Pathology and Microbiology and Immunology, University of Texas Medical Branch, Galveston, Texas.

Rift Valley fever virus (RVFV) (genus *Phlebovirus*, family Bunyaviridae) is an arthropod-borne virus with tripartite, single stranded, negative sense RNA genomes. RVFV, primarily transmitted by mosquitoes, has been responsible for large explosive epizootics/epidemics of severe animal and human disease throughout Africa. A number of these outbreaks have occurred in areas where the virus has historically not occurred in the past, initiating large virgin-soil epizootics/epidemics affecting both the human and livestock populations in a significant manner. Even in areas where the virus is known to be endemic, the extended period between periodic epizootics/epidemics is sufficient to create high susceptibility in the domestic livestock that serve as the principal amplifying hosts for the virus. RVFV vector populations are governed by variable rainfall patterns in the endemic areas and use of remote sensing to attempt to predict the epizootics/epidemics is an area of active research. The necessity of multidisciplinary approaches to outbreak investigation and research on the virus is self-apparent; entomologists, animal health and public health laboratorians and epidemiologists all play large roles in understanding the underlying ecology and the epidemic emergence of the disease. Examples from outbreaks in Egypt in 1977 and the Arabian peninsula in 2000 will be used to illustrate the impact in previously unaffected areas and outbreaks in Kenya, Tanzania, Somalia in 1997-8 and 2006-7 will be used to illustrate the still impressive impact in endemic areas of East Africa.

3:00 PM

Bovine tuberculosis: Immune response and vaccine efficacy studies. W. R. Waters, M. V. Palmer, T. C. Thacker, National Animal Disease Center, Ames, IA

Bovine tuberculosis (TB) is a re-emerging disease of cattle within the United States, primarily due to importation of infected cattle from Mexico and the emergence of a wildlife reservoir (white-tailed deer) in Michigan. While the mainstay of bovine TB control has been abattoir inspection plus targeted test/cull campaigns, vaccines are now being considered as an additional tool for control, both in cattle and wildlife. To evaluate candidate TB vaccines, a neonatal calf model was developed to objectively measure efficacy and immunogenicity. Calves are vaccinated at ~2 wks of age and virulent *M. bovis* delivered by aerosol at ~3.5 months of age. Variables used to evaluate vaccine efficacy include: gross pathology, radiograph morphometry, histopathology, mycobacterial culture (quantitative and qualitative), and various immune parameters. A similar protocol was developed for evaluation of vaccine efficacy in WTD; however, the vaccine is administered to young adults (~1 yr of age) and *M. bovis* is delivered by instillation into the tonsillar crypts. For calves, vaccination with *M. bovis* $\Delta RD1$ (the primary attenuating defect of *M. bovis* BCG) induced protection equivalent to BCG. Mean central memory T cell responses elicited by either *M. bovis* $\Delta RD1$ or BCG prior to challenge correlated with reduced pathology and bacterial colonization. Reduced in vitro recall responses after challenge were also associated with effective $\Delta RD1$ and BCG vaccines. Vaccination of white-tailed deer with BCG by either subcutaneous or oral routes resulted in reduced *M. bovis*-associated pathology compared to non-vaccinates; however, BCG persisted within tissues up to 250d, disseminated to distant lymphoid tissues, and transmitted from deer to deer. In addition to vaccine efficacy studies, this review highlights recent advances in our understanding of the immunopathogenesis of bovine TB.

3:45 PM

The Role of the Pig in Pandemic Flu. Jürgen A. Richt, DVM, PhD. Regents Distinguished Professor and Director, Center of Excellence for Emerging and Zoonotic Animal Diseases (CEEZAD), Kansas State University, College of Veterinary Medicine, Manhattan, KS 66502, USA.

Influenza is a viral disease with remarkable zoonotic potential and therefore represents a significant threat to the health of both humans and a variety of animal species worldwide. Swine influenza (SI) was first recognized clinically in pigs in the Midwestern U.S. in 1918 coinciding with the human influenza pandemic known as the "Spanish flu". Since that time SI has remained of importance to the swine industry throughout most of the world.

In this presentation, the epidemiology of SI virus (SIV) infections in North American pigs in the last 90+ years is discussed. For the first 80 years, SIV evolution remained relatively static, whereas in the last decade SIV evolution has become dynamic with the establishment of many novel emerging subtypes culminating in the appearance of the pandemic H1N1 virus in the spring of 2009. The gene constellation of the 2009 pandemic A/H1N1 (pH1N1) virus is a unique quadruple reassortment combination most likely derived from SIVs of North American (triple reassortant SIVs) and Eurasian (avian-like SIVs) lineages; prior to April 2009 the genetic constellation of the pH1N1 virus had never before been identified in swine or other species.

Acknowledgments: Grant support from Department of Homeland Security (CEEZAD), NIAID, NIH, Department of Health and Human Services (Contract No. HHSN266200700005C) and the Centers for Disease Control and Prevention (Grant No. U01 CI000357-01).

Animal Health Surveillance: From Science to Application
– A Symposium Honoring the Professional Legacy of Dr. Preben Willeberg –



The Association of Veterinary Epidemiology and Preventive Medicine (AVEPM) is pleased to announce the program for the 2010 Schwabe Symposium honoring the professional achievements of Dr. Preben Willeberg. The symposium will be held in Chicago on [Sunday, Dec 5, 2010](#), 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 **Introductory remarks**

12:35 pm **Global and Regional Surveillance of Bovine Spongiform Encephalopathy: Their impact on reducing the spread of the disease**

Mo Salman, Professor of Veterinary Epidemiology, Colorado State University

1:15 pm **Return on Investment of National Animal Health Surveillance**

Aaron Scott, Center Director, National Surveillance Unit, USDA/APHIS/VS/CEAH

1:55 pm **Four elements of surveillance - science, project management, communication and documentation**

Jette Christensen, Epidemiologist, Canadian Food Inspection Agency

2:35 pm Break and Refreshments

2:55 pm **The consequences of freedom: Developing output-based standards for surveillance to demonstrate freedom from disease**

Angus Cameron, Director, AusVet Animal Health Services

Keynote address:

3:35 pm **Animal health surveillance applications: The interaction of science and management**

Preben Willeberg, Senior Veterinary Global Health Specialist, Center for Animal Disease Modeling and Surveillance (CADMS), School of Veterinary Medicine, UC Davis

4:30 pm Reception / meet the speakers (refreshments and cash bar)

5:15 pm Adjourn

6:00 – 8:00 pm CRWAD Researchers Reception and Poster Viewing

The Calvin W. Schwabe Award is presented annually by the AVEPM to honor lifetime achievement in veterinary epidemiology and preventive medicine. The 2010 honoree is:

Preben W. Willeberg, DVM, PhD, DVSc, Dr.med.vet.h.c., Dip. ECVPH

Dr. Preben Willeberg is a Senior Veterinary Global Health Specialist with the Center for Animal Disease Modeling and Surveillance at the School of Veterinary Medicine, University of California, Davis, Adjunct Honorary Professor of Animal Disease Control and Surveillance at the University of Copenhagen, and Adjunct Honorary Professor of Epidemiology at the Technical University of Denmark. Throughout his career, he has been highly respected as a mentor and leader in the fields of veterinary epidemiology and preventive medicine, particularly in animal disease surveillance and management. Dr. Willeberg earned his DVM (1967), PhD (1972), and DVSc. (1977) from the Royal Veterinary and Agricultural University in Copenhagen, Denmark. Dr. Willeberg joined the faculty at the Royal Veterinary and Agricultural University as an Associate Professor in 1974, and was promoted to Professor of State Veterinary Medicine and Epidemiology in 1985. In 1995, he was awarded the honours degree of Doctor of Veterinary Medicine *honoris causa* by the Faculty of Veterinary Medicine at the University of Helsinki in Finland. From 1999 to 2007, he was the Chief Veterinary Officer for Denmark, during which time he was responsible for managing outbreaks of Bovine Spongiform Encephalopathy (BSE) (2000-2005), Exotic Newcastle Disease (END) (2002), and Highly Pathogenic Avian Influenza (HPAI) (2006) in Denmark. He is a Diplomate and Founding Member of the European College of Veterinary Public Health.

Dr. Willeberg is the co-author of the 1987 textbook: *Veterinary Epidemiology – Principles and Methods* with Wayne Martin and Alan Meek, and he has been an author on more than 100 papers published in peer-reviewed journals. He has served on various expert scientific committees throughout his career, organized the 5th International Symposium on Veterinary Epidemiology and Economics (ISVEE) in Copenhagen (1988), and has been a visiting professor at several universities. Dr. Willeberg was chief editor of *Acta Veterinaria Scandinavica* from 1981-1992, and has also served on the editorial boards of *Preventive Veterinary Medicine* (1982-1990), *Veterinary Research Communications* (1977-2003), and *Compendium on Continuing Education for the Practicing Veterinarian* (1991-1994). He has been active in international health organizations, including serving as the Danish Delegate to the FAO European Commission for the Control of Foot-and-Mouth Disease (EUFMD) 1999 – 2007, elected as an Executive Committee member (three terms), and serving as Vice-President from 2003 – 2004 and the Liaison Officer to the Permanent Research Group from 2004 – 2007. Dr. Willeberg was the Danish Delegate to the World Animal Health Organization (OIE) from 1999 – 2007, was elected as Secretary General of the OIE Scientific Commission for Animal Diseases from 2006 – 2009 and has been a member of several OIE Ad Hoc Working Groups. He has been decorated three times by Her Majesty the Queen of Denmark for his services to the Danish Government, lastly in October 2007 as “Commander of the Order of Dannebrog”.

2010 International PRRS Symposium

December 3 and 4, 2010

2010 IPRRSS Organizing Committee:

Chair: X.J. Meng
Virginia Polytechnic Institute and State University
Co-Chair: Lisa Becton
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Jenny Welch, Pfizer Animal Health
Dongwan Yoo, University of Illinois

The 2010 International PRRS Symposium Program has been approved for 12 hours of continuing education (CE) credit in jurisdictions which recognize AAVSB RACE approval for veterinarians and veterinary technicians.

Confirmation forms will be available at the PRRS Symposium Registration Desk; signed CE forms must be submitted at the registration desk before the end of the Symposium.

Keynote presentations: 60/50 minute total with a 50/40 minute oral presentation and a 10 minute interactive discussion.

Oral presentations: 20 minutes total with a 15 minute oral presentation and 5 minutes interactive discussion

Friday, December 3rd

14:00-17:30 Symposium Registration (5th floor registration booth)

Pre-Symposium Meetings-Open to public

13:00-14:30 ***Business meeting Multistate Research Project NC-229***
Detection and Control of Porcine Reproductive and Respiratory Syndrome Virus and Emerging Viral Diseases of Swine.

Chair: X.J. Meng

Secretary: Jane Christopher-Hennings

Advisor: David Benfield

13:00 **X.J. Meng**, Opening remarks

13:05 **David Benfield**, NC-229 history and evolution

13:10 **Peter Johnson and Margo Holland**, USDA-NIFA; USDA funding opportunities and changes

13:20 **Bob Rowland**, Post-PRRS-CAP funding for NC-229, and discussion with NC-229 members

13:30 **Short Station Updates**

14:30 **Jane Christopher-Hennings**, Closing

14:30-16:00 ***PRRSV Elimination Workshop/USDA CAP-2 meeting***
Bob Rowland, Director

Impact of transportation on disease control and eradication programs

Bob Morrison, Chair; Jeff Zimmerman, Co-Chairs

Keynote lectures:

Overview of disease control and eradication, and role of transportation.

Jeff Zimmerman, Iowa State University

Implications of transportation for controlling disease within a large integrated production system.

Spencer Wayne, Pipestone System and Pipestone Veterinary Clinic

Outbreak scenarios from the perspective of animal movement and what the impact on outbreak magnitude would be if we were to make modifications in frequency and or quantity of animals transported.

Celia Antognoli, USDA National Surveillance Unit

Round-table discussion

2010 International PRRS Symposium

Opening Session

X.J. Meng, Chair, and Lisa Becton, Co-Chair

- 16:00 Welcome. **X. J. Meng**, Chair – 2010 International PRRS Symposium
16:10 **Keynote Presentation #1:**
Ralph Baric, *Univ. of North Carolina at Chapel Hill,*
Topic: Nidovirus Vaccine Design: Novel Opportunities and Challenges
- 17:15 Reception and cash bar
First Poster Session until 19:00

Saturday, December 4th

Session I: Molecular Biology: Structural and Functional Relationships

Co-Chairs: Dongwan Yoo & Jenny Welch

- 8:00 **Keynote Presentation #2:**
Eric Snijder, *Leiden Univ. Medical Center, The Netherlands*
Topic: The arterivirus replicase: Exploring the multifunctionality of an intriguing set of nonstructural proteins
- 8:40 The cysteine protease domain of PRRSV nonstructural protein 2 possesses deubiquitinating and interferon antagonism functions.
Ying Fang, *South Dakota State University, Brookings, SD.*
- 9:00 Porcine Reproductive and Respiratory Syndrome Virus-Associated Glycans are Potential Targets for Virus Neutralization.
Juan Li, *University of Minnesota, Minneapolis, MN.*
- 9:20 Porcine reproductive and respiratory syndrome virus E protein is an integral membrane protein with type-II₆ orientation and colocalizes with GP2, GP3, GP4 and M protein.
Wenjun Liu, *Chinese Academy of Sciences, Beijing, China.*
- 9:40 **Coffee break** (refreshments in the poster area)
Second Poster session

Session II: Virus-Host Interaction and Pathogenesis

Co-Chairs: Tanya LeRoith & Kelly Lager

- 10:40 Three amino acids of ORF5 are jointly responsible for virulence of porcine reproductive and respiratory syndrome virus.
Byungjoon Kwon, *University of Nebraska, Lincoln, NE.*
- 11:00 ORF1b contributes to the increased fatal virulence of highly pathogenic porcine reproductive and respiratory syndrome virus emerging in China.
Hanchun Yang, *China Agricultural University, Beijing, China.*

- 11:20 Interaction of PRRSV Nsp1a and protein inhibitor of activated STAT1 (PIAS1) mediates sumoylation of Nsp1a.
Dongwan Yoo. University of Illinois, Urbana, IL.
- 11:40 Regulation of interferon type I response of porcine plasmacytoid dendritic cells by cytokines and viruses.
Artur Summerfield. Institute of Virology and Immunoprophylaxis, Mittelhausern, Switzerland.
- 12:00 **Lunch buffet in the poster area**

Session III: Vaccine and Immunity
Co-Chairs: Dick Hesse & Wen-hai Feng

- 13:00 Replication Competent Recombinant PRRS Viruses Expressing Indicator Proteins and Antiviral Cytokines.
Yongming Sang. Kansas State University, Manhattan, KS.
- 13:20 Porcine Reproductive and Respiratory Syndrome Virus (PRRSV) isolates differ in their susceptibility to neutralization by a battery of monospecific PRRSV antisera.
Cinta Prieto Suarez. Universidad Complutense de Madrid, Madrid, Spain.
- 13:40 Absence of glycan moieties in GP3 & GP5 of a PRRSV field isolate enhances its susceptibility to antibody neutralization and its ability to elicit neutralizing antibody response.
Hiep Vu. University of Nebraska, Lincoln, NE.
- 14:00 Immune and genetic control of swine responses to porcine reproductive and respiratory syndrome virus infection.
Joan K. Lunney. USDA ARS BARC, Beltsville, MD.
- 14:20 Mucosal immunization with biodegradable PLG- nanoparticles elicits effective anti-PRRSV immune responses in pigs.
Varun Dwivedi. Ohio State University, Wooster, OH.
- 14:40 **Break** (refreshments in the poster area)

Session IV: Epidemiology, Heterogeneity and Evolution
Co-Chairs: Tomasz Stadejek & Derald Holtkamp

- 15:10 Weapons of mass-reduction: applying network statistics to define core PRRSV sequences for rational polyvalent vaccine development.
Tavis Anderson. University of Wisconsin, Madison, WI.
- 15:30 Molecular epidemiology of porcine reproductive and respiratory syndrome virus (PRRSV) in Ontario, Canada.
Manreetpal S. Brar. University of Hong Kong, Hong Kong, China.
- 15:50 Pen-based oral fluid sampling for PRRSV using an optimized PRRSV PCR assay is highly effective for the detection of virus in low prevalence populations.
John Prickett. Iowa State University, Ames, IA.
- 16:10 Sensitivity, specificity and safety of FTA cards inoculated with PRRS virus.
Daniel Linhares. University of Minnesota, Minneapolis, MN.
- 16:30 Control of PRRSV in Vietnam - more systematic and specific approaches are needed.
Nguyen Van Dien. Bayer Vietnam, Ltd., Ho Chi Minh, Vietnam.
- 16:50 **Closing Remarks**
Jane Christopher-Hennings, Scientific Program Chair.
- 17:00 **Adjourn**

POSTER ABSTRACTS

BACTERIAL PATHOGENESIS POSTERS

001P

Use of Transposon Mutagenesis and Microarray Analysis to identify genes associated with biofilm formation in *Actinobacillus pleuropneumoniae*.
Y. Tremblay, A. Grasteanu, M. Jacques, Université de Montréal, St-Hyacinthe, QC, Canada, Email: mario.jacques@umontreal.ca.

Purpose: *Actinobacillus pleuropneumoniae* is the Gram-negative bacterium responsible for porcine pleuropneumonia. This respiratory infection is highly contagious and characterized by high morbidity and mortality rates. In some cases, *A. pleuropneumoniae* infection can develop into a chronic disease. Pigs suffering from a chronic infection are considered to be carriers and these carriers likely play an important role in transmission. In several bacterial infections it is known that persistent chronic infections are characterized by the presence of a biofilm. Furthermore, it was recently demonstrated that clinical isolates and reference strains of *A. pleuropneumoniae* can form biofilms. Therefore, it was hypothesized that *A. pleuropneumoniae* forms biofilms during the infection process. The objective of this study was to identify genes associated with biofilm formation.

Methods: Two approaches were used to accomplish the objective. The first approach used transposon mutagenesis analysis of *A. pleuropneumoniae* serotype 1 strain S4074, the subsequent transposon library was screened to identify mutants with increased or decreased ability to form biofilms in 96-well polystyrene plates. In the second approach, the transcript profiles of 6 hour biofilm cells was compared to the transcript profiles of 6 hour planktonic cells using an *A. pleuropneumoniae*-specific microarray.

Results: The transposon mutagenesis analysis identified 78 mutants that exhibited abnormal biofilm formation, at least 32 unique genes were identified. Most genes identified in the enhanced-biofilm mutants encoded proteins related to the cell-envelope, whereas most genes identified in the biofilm-negative mutants encoded proteins related to transport and metabolism. Microarray analysis flagged 49 up-regulated genes and 68 down-regulated genes in the 6 hour biofilm. The down-regulated genes were mostly associated with energy metabolism, whereas the up-regulated genes were mostly associated with transporters. Interestingly, 5 genes were identified in both analyses.

Conclusions: Both approaches allowed the identification of new genes associated with biofilm formation in *A. pleuropneumoniae*.

002P

A comparative and functional genomics glimpse of a highly virulent strain of *Campylobacter jejuni* associated with sheep abortion.
Z. Wu, O. Sahin, Q. Zhang, Department of Veterinary Microbiology and Preventive Medicine, Iowa State University, Ames, IA, Email: wuzw@iastate.edu.

Purpose: A highly virulent *Campylobacter jejuni* strain (IA3902) has emerged as the major cause of sheep abortion in the United States. This study is aimed to uncover the pathogenic mechanisms of IA3902.

Methods: We sequenced its genome and initiated comparative and functional genomics studies to determine its unique genetic attributes.

Results: The genome of IA3902 contains a single circular chromosome of 1,635,045 bp and a circular plasmid of 37,174 bp. Phylogenetic and comparative genomic analysis showed that the genome of IA3902 is closely related to other sequenced *C. jejuni* strains, especially to strain NCTC 11168. Despite the high genomic synteny and similarity, there are 29 unique genes in IA3902 and several thousands of SNPs between the genome of IA3902 and other strains. Comparative transcriptomic analysis revealed that 133 genes were up-regulated and 129 were down-regulated in IA3902 compared to NCTC 11168. KEGG pathway analysis showed that the up-regulated genes are significantly associated with flagellar assembly, bacterial motility and energy metabolism, while the down-regulated genes are mainly involved in transport functions. Proteomic analysis using 2D-DIGE identified more than 90 protein spots that are significantly different between IA3902 and 11168. Spot identification revealed that most of the differences in protein spots were due to sequence polymorphisms that resulted in shifts in size or PI of proteins. However, 18 proteins were identified to be truly different between IA3902 and 11168, with 6 over-expressed and 12 down-expressed in IA3902. A good correlation was observed between the protein data and the microarray data, and both methods revealed that several iron uptake systems were down-expressed in IA3902.

Conclusions: These results provide a preliminary global view on the differences between IA3902 and 11168 at the genomic, gene transcription, and protein expression levels and will facilitate the design of future studies to dissect the pathogenic mechanisms of *C. jejuni* IA3902.

003P

Identification of immunogenic insoluble proteins of *Brucella abortus* separated by two-dimensional electrophoresis to develop specific antigens required for Brucellosis diagnosis.

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Purpose: In order to overcome the limitations of serological diagnosis of brucellosis including false positive reactions, the development of specific antigens for besides LPS have been required. The present study was to search for specific antigens in insoluble proteins of *Brucella abortus* 1119-3 to reduce the cross reactions caused by other strains such as *Yersinia enterocolitica*.

Methods: After culturing *B. abortus* at 37°C for 24-48 h under shaking, the collected cells were destroyed by French press and after centrifugation the pellet obtained was sufficiently solubilized with lysis buffer and the supernatant was represented to be insoluble proteins in the present study. These proteins were completely separated on 2-DE and their immunologic reactivity were determined by western blotting. Among these immuno-dominant spots on western blotting, those having immunogenic reactivity against only *B. abortus*-specific hyperimmune sera, not *Y. enterocolitica*-specific hyperimmune sera and the sera obtained from normal cattle, were selected and identified for using as candidates of the useful specific antigens.

Results: As a result, among 39 spots having immunogenic reactivity against *B. abortus*-specific hyperimmune sera, only 18 spots were selected as the specific antigen candidates because they indicated to have immunogenic reactivity against only *B. abortus*-specific hyperimmune sera, but not other sera. According to MS/MS analysis for their identification, they were disclosed to appear a variety of proteins such as Leu/Ile/Val-binding family protein, histidinol dehydrogenase, ATP synthase subunit beta, solute-binding family 5 protein, twin-arginine translocation pathway signal sequence domain-containing protein, serine protease family protein, β -hydroxyacyl-(acyl-carrier-protein) dehydratase FabA, and superoxide dismutase.

Conclusions: The present study suggests that these immuno-dominant proteins seem to have potential as specific antigens, which can reduce false positive reactions by other strains in the diagnosis of Brucellosis.

004P

Development of the improved multiplex PCR assay for the differential identification of *Brucella* species

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BACTERIAL PATHOGENESIS POSTERS

004P (continued)

Brucellosis is a zoonotic disease caused by facultative intracellular pathogens. *Brucella* species have been divided with 9 different species by the range of strain host. The *Brucella* species have highly homology of the genetic similarity, except for the marine mammal's species. Many studies have been carried out a long time in order to develop PCR-based assays for the discrimination among species of brucellae. However, neither tested for the identification for *B. suis* strains from some of *B. canis* strains nor for the differentiation between the marine mammal's *B. ceti* and *B. pinnipedialis* by the multiplex-PCR profiles.

To discriminate the *B. suis* and *B. canis* strains, the deleted genetic site of *B. canis* strain founded out through the alignments for their whole genome sequence. The specific primer for the deleted genetic site was designed by the Primer-BLAST of the Pubmed site and their amplification size was designed as 766 bp, which is to replace 794 bp of the Bruce-ladder PCR assay described previously. To confirm specific amplification of this primer, sequence analysis for this genetic site of the *Brucella* species including *B. canis* isolates was performed and the deletion of primer site from all *B. canis* strains tested is confirmed.

All *B. suis* reference strains (biovar 1-5) was amplified by this primer, but *B. canis* reference strain and *B. canis* isolates were not detected at all. The improved multiplex-PCR assay replaced to the new designed primer sets can discriminate between *B. suis* strains and *B. canis* strains including isolates identified as *suis*-like strains by previous assay. Moreover, two marine *Brucella* reference strains (*B. ceti* and *B. pinnipedialis*) were clearly distinguished by sequence analysis of this site. *B. abortus* and *B. neotomae* have also been showed to change.

Consequently, the improved multiplex PCR assay can differentiate all *Brucella* species except for *B. microti*.

005P

Gene expression profile of bovine primary mammary epithelial cells infected with *Escherichia coli* associate with acute or chronic bovine mastitis.

O. Kerro Dego, R. Almeida, D. Luther, S. Oliver, The University of Tennessee, Knoxville, TN, Email: okerrode@utk.edu.

Acute *E. coli* mastitis is usually associated with rapid onset of clinical signs including mammary gland swelling, abnormal milk, and rapid clearance of bacteria from milk. In some cases, severe systemic involvement occurs which could result in death of the animal. Some strains of *E. coli* are associated with chronic mastitis which often times begins with mild clinical symptoms that disappear soon after onset of infection only to flare-up again during lactation, resulting in mild clinical mastitis. Recently published data suggests that adhesion to and internalization into bovine mammary epithelial cells and subsequent intracellular survival might be important virulence attributes of strains of *E. coli* associated with chronic mastitis. The aims of this study were to compare gene expression profiles of both eukaryotic (primary bovine mammary epithelial cells-PBMEC) and prokaryotic (acute and chronic *E. coli* strains) co-cultured for 1 h and to identify genes of PBMEC and virulence factors of *E. coli* involved in internalization into and intracellular persistence in mammary epithelial cells. The expression patterns of virulence genes of these strains of *E. coli* have been evaluated by qRT-PCR. Our results showed that gene expression patterns in PBMEC infected with acute or chronic strains were significantly different from that of uninfected cells. Expressions of genes in PBMEC infected with the acute strain were higher than that of PBMEC infected with the chronic strain. Genes that showed increased expression of transcript were primarily pro-inflammatory cytokines and complement. The chronic strain of *E. coli* induced lower cell activation than the acute strain of *E. coli*, thus explaining mild clinical symptoms observed in natural chronic *E. coli* IMI and increased intracellular persistence of chronic *E. coli* strains in vitro.

006P

Immune response following vaccination of dairy cows with recombinant *Streptococcus uberis* adhesion molecule (rSUAM).

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Research conducted in our laboratory on the pathogenesis of *Streptococcus uberis* mastitis lead to the discovery of a novel virulence factor, referred to as SUAM. Further research showed that antibodies directed against rSUAM inhibited adherence to and internalization of *S. uberis* into mammary epithelial cells, placing rSUAM as a promising antigen for the control of *S. uberis* mastitis. To design an effective vaccine formulation, we compared the safety and humoral immune response (IgG) induced by rSUAM emulsified with commercially available adjuvants (Emulsigen, Seppic VG70, Seppic ISA760, and Seppic ISA780). For this experiment, 5 cows per each formulation and a control group ($n = 4$) were vaccinated 28 days before dry off (D-28), at dry off (D0) and 28 days after dry off (D+28). Serum samples were obtained every 2 weeks from D-28 to 28 days after calving (C+28). Rectal temperature and local reaction measurements were obtained 3 times during the 1st week and every 2 weeks after each subsequent vaccination. Result obtained showed that IgG response induced by SV70-rSUAM was significantly higher than the control (rSUAM alone) or the rest of the vaccine formulations tested. Local reaction induced by SV70-rSUAM was not different than the rest of the vaccine formulations with the exception of the second vaccination, where the local reaction of SV70-rSUAM was significantly higher than the rest. Results of this study lead to the selection of SV70-rSUAM for an upcoming vaccination study which includes challenge of vaccinated and control cows with homologous and heterologous *S. uberis* strains.

007P

Cross-reactivity of antibodies against leptospiral recurrent uveitis-associated proteins with ocular proteins.

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Infection by *Leptospira* interrogans has been causally associated with human and equine uveitis. Studies in our laboratory have demonstrated that leptospiral lipoprotein LruA and LruB are expressed in the eyes of uveitic horses, and that antibodies directed against LruA and LruB react with equine lenticular and retinal extracts, respectively. These reactivities were investigated further by performing immunofluorescent assays on lenticular and retinal tissue sections. Incubation of lens tissue sections with LruA-antiserum and retinal sections with LruB-antiserum resulted in positive fluorescence. By employing two-dimensional gel analyses followed by immunoblotting and mass spectrometry, lens proteins cross-reacting with LruA antiserum were identified to be α -crystallin B and vimentin. Similarly, mass spectrometric analyses identified β -crystallin B2 as the retinal protein cross-reacting with LruB-antiserum. Purified recombinant human α -crystallin B and vimentin were recognized by LruA-directed antiserum, but not by control pre-immune serum. Recombinant β -crystallin B2 was likewise recognized by LruB-directed antiserum, but not by pre-immune serum. Moreover, uveitic eye fluids contained significantly higher levels of antibodies that recognized α -crystallin B, β -crystallin B2 and vimentin than did normal eye fluids. Our results indicate that LruA and LruB share immuno-relevant epitopes with eye proteins, suggesting that cross-reactive antibody interactions with eye antigens may contribute to immunopathogenesis of *Leptospira*-associated recurrent uveitis.

BACTERIAL PATHOGENESIS POSTERS

008P

Immunization with recombinant *Brucella abortus* outer membrane protein Omp25d, reduces bacterial load after challenge in a murine model for brucellosis.

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Purpose: We previously identified a *Brucella abortus*, Type-V auto-secreting protein up-regulated during infection in elk (Lowry, et. al 2010) which is orthologous to the virulence factor, Omp25d, in *B. ovis*.

Methods: To examine the role of Omp25d in the stimulation of protective immunity against *B. abortus*, we first PCR-amplified the locus from RB51 genomic DNA, then cloned and stably expressed the recombinant product from *E. coli* as a 208 amino acid- truncate. After purification as a histidine-tagged fusion protein, Omp25d was adjuvanted with aluminum hydroxide and used to immunize BALB/c mice. Three weeks after the last vaccine boost, animals were challenged with 5×10^4 CFU of either *B. abortus* S19 or 2308, by ip. route.

Results: Despite variably low antibody levels to the protein, by 7 days post-infection the 2308 challenged Omp25d immune mice exhibited a significant reduction in bacterial load in their spleens (1.03 logs; $p=0.016$), while S19-challenged mice were no different than the adjuvant-only controls. Clearance in the 2308 challenged Omp25d-immunized animals was also comparable to that seen in animals vaccinated with *B. abortus* RB51. Immunization with an antigenic recombinant protein from an unrelated pathogen prepared identically to Omp25d had no effect on clearance of the *B. abortus* challenge. At day 14 post-infection, splenic bacterial loads were reduced in both Omp25d immune groups, ranging from 0.50 (S19; $p < 0.01$) to 0.73 log units (2308; $p=0.05$), compared with the adjuvant-only groups. Additionally, S19-challenged, Omp25d-vaccinated mice generated persistently elevated INF- γ levels relative to the control group at day 14 ($p < 0.05$), correlating with reduced colonization in the spleen.

Conclusions: Taken together, these data suggest that immunity to Omp25d may offer some protection during *B. abortus* infection through a pro-inflammation stimulatory process, and thus represents a possible vaccine candidate for further study in domestic/wildlife species.

009P

MAP induces extracellular calcium dependent phagosome acidification to enlist IL-1 β processing and macrophage recruitment.

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Purpose: Processing of MAP by the host epithelium involves a dynamic innate immune response initiated by MAP-epithelial cell cross-talk, which may also be further augmented by interactions between host pathways and/or cell types (epithelial-macrophage). Methods and Results: We show that MAP induces phagosome acidification within MacT cells as early as 10 min., resulting in upregulation of IL-1 β at transcript and protein levels. Previous studies report that IL-1 β is a potent macrophage chemoattractant. We hypothesized that MAP harnesses host responses to recruit macrophages to the site of infection to insure its survival and dissemination. These initial host-pathogen interactions may dictate a form of cooperative self-destruction in which the host is deceived into reacting to the benefit of MAP; thereby, setting the tone for the ensuing infection. We investigated macrophage recruitment in response to MAP using a MacT-bovine macrophage co-culture system. Within 10 min of MAP infection, macrophages were recruited to the apical side of Mac-T cells. Inhibiting phagosome acidification with bafilomycin treatment abrogated this response. Since IL-1 β cleavage and subsequent release to the epithelial milieu is dependent upon calcium influx, we next sought to define the role of calcium oscillations in phagosome acidification and macrophage recruitment. Pre-treatment of Mac-T cells with BAPTA-AM, an established intracellular calcium chelator, abolishes phagosome acidification and IL-1 β processing to its active form. Removal of calcium from media using EGTA results in loss of phagosome acidification, indicative of an extracellular calcium flux. These data were validated with supplementing calcium free media with uridine triphosphate (UTP), an established agonist of purinergic receptors and intracellular calcium flux, which restored phagosome maturation function. Conclusions: Thus, MAP guidance of phagosome-acidification enlists IL-1 β processing in a extracellular calcium dependent manner in order to efficiently transverse the epithelium and into its niche_the macrophage.

010P

Enteroaggregative *Escherichia coli* heat-stable toxin 1 (east1) is not sufficiently virulent to cause diarrhea in neonatal pigs.

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Purpose: Enterotoxigenic *Escherichia coli* (ETEC) strains are the major cause of diarrhea disease in neonatal and weaned pigs. *E. coli* fimbriae and heat-labile (LT) and heat-stable (ST) enterotoxins are the key virulence factors in porcine ETEC diarrhea. Epidemiology studies indicated that enteroaggregative heat-stable toxin 1 (EAST1), heat-stable b (STb), LT and heat-stable a (STa) toxins are highly prevalent in *E. coli* strains isolated from pigs with diarrhea. While LT, STa and STb are proven to be the virulence determinants in ETEC associated diarrhea in pigs, the role of EAST1 in porcine diarrhea has never been unambiguously determined. Methods: In this study, we cloned *astA* into vector pUC19 to generate an *E. coli* strain that expresses EAST1.

Furthermore, we expressed K88ac fimbria in enteroaggregative *E. coli* (EAEC) strain O-42. 7-day old gnotobiotic pigs were inoculated with these two strains to assess the significance of EAST1 in porcine diarrhea.

Results: During 72 h postinoculation, none of the challenge piglets developed clinical diarrhea or dehydration. In addition, *in vitro* studies indicated that culture supernatant of EAST1-positive *E. coli* strains did not stimulate an increase of intracellular cyclic AMP or GMP in T-84 cells or the porcine cell line IPEC-J2, nor did EAST1 containing culture supernatant provided additive role to LT in the stimulation of cAMP or STa stimulation of cGMP.

Conclusions: Data from our studies suggest that EAST1 may not be sufficiently virulent to cause diarrhea in neonatal pigs, and provides information for porcine ETEC vaccine development.

011P

Protective efficacy of a *Francisella tularensis* type A complemented O-antigen mutant against murine tularemia.

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Purpose: *Francisella tularensis* is the cause of tularemia in a wide variety of mammals. Mutants of *Francisella tularensis* lacking lipopolysaccharide (LPS) O-antigen are highly attenuated in mice, and therefore may be vaccine candidates.

Methods: Following mutagenesis of a highly virulent feline type A isolate (strain TI0902), a base substitution in *wbtK* in the O-antigen locus (encoding for a glycosyltransferase) was identified by sequencing. Complementation of the O-antigen with *wbtK* *in trans* resulted in 20 complemented isolates, which were further characterized by Western blotting, serum resistance, and virulence in mice. Blood and tissue cytokine levels were determined by ELISA.

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011P (continued)

Results: This mutant produced no O-antigen, was serum-susceptible and highly attenuated, but was unable to protect mice against TI0902 challenge. The O-antigen and serum resistance was restored in all complemented strains examined. However, some complemented strains remained avirulent following intradermal (ID) challenge with $> 5 \times 10^7$ cells, while others regained full virulence. ID immunization of mice with 10^7 cells of an avirulent, complemented strain protected all mice from morbidity and mortality against ID challenge with 3.8×10^7 TI0902 cells 2 weeks and 3 months post-immunization. In addition, ID immunization with 10^5 cells protected over 50% of mice challenged intranasally with 10^3 cells of TI0902. Sera collected from mice immunized with the protective complemented mutant one week postchallenge contained significantly more ($P < 0.01$) IFN- γ than mice immunized with the non-protective O-antigen mutant. Furthermore, over 60% of mice immunized with the O-antigen mutant mixed with purified *F. tularensis* LPS conjugated to keyhole limpet hemocyanin were protected against challenge with strain type A strain Schu S4, and there was a marked increase in IFN- γ in these mice, though less than in mice immunized with the complemented mutant.

Conclusions: Thus, adequate levels of IFN- γ following immunization may be a useful marker of protection against *F. tularensis*, and type A O-antigen mutants that are complemented *in trans* and remain attenuated may be vaccine candidates against *F. tularensis* type A.

012P

The *invA* gene (BMEI0215) of *Brucella melitensis* is needed for intracellular replication, but not for invasion.

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Purpose: *Brucella melitensis* is an intracellular microorganism that causes economic losses in developing countries, affecting their dairy industry. It is relevant in public health, being the most important bacterial zoonosis in Mexico. The use of the Rev1 vaccine in goats and sheep is effective, preventing the infection in the field, but it has residual virulence for animals and humans. Therefore there is a need for better vaccines against *Brucella melitensis*. Methods: The objective of this work was to evaluate the *Brucella melitensis invA*-km mutant, in the macrophage and epithelial cells invasion models, and compared them with the Rev1 vaccine, the parental strain 133 and *Brucella melitensis* 16M. We used the J774A.1 macrophage cell line and the Hela epithelial cell line.

Results: In the macrophage model the mutant replicated less than the parental strain at 24 and 48 hrs post-infection ($P < 0.001$). There was no statistically difference in the survival between the mutant and the parental strains at 0 and 4 hrs. post-infection. The genetic complementation of the mutant was done *in trans* with the plasmid pBBRMCS4.1. No significant differences were evident neither on survival nor replication processes between the mutant and the complemented mutant strain (*Brucella melitensis* 133 *invA*-km⁺). The survival and replication of the Rev1 and 16M strains were not statistically different from the mutant and complemented strains ($p > 0.05$). Similar results were obtained with peritoneal murine macrophages. On the other hand, epithelial Hela cells invasion was lower ($P < 0.001$) for the Rev1 strain at 0 and 1 hr post-infection than the mutant, parental and 16M strains of *Brucella melitensis*. The complemented mutant strain had an invasion behavior similar to the Rev1 strain at 0 hr with no statistically significant differences ($p > 0.05$), but not at 1 hr post-infection ($P < 0.001$).

Conclusions: These results show that the mutation on the *invA* gene affected the replication in macrophages, but is not crucial on the survival and/or invasion processes. This work was supported by the Universidad Nacional Autónoma de México (grant PAPIIT-UNAM No. IN207608)

013P

Citrobacter rodentium causes structural and functional alterations in conditionally immortalized Ptk6 colonic epithelial cells.

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Citrobacter rodentium is a highly virulent enteric pathogen of mice, and is an animal model for enteropathogenic and enterohemorrhagic *E. coli* infections of humans. In mice, *C. rodentium* attaches to apical surface of the colonic epithelium resulting in the effacement of the microvilli, enterocyte hyperplasia, and rectal prolapse. The objective of this study was to develop a tissue monolayer model for *C. rodentium* infection in mice. A conditionally-immortalized colonic epithelial cell line derived from a *Ptk6* null mouse was used in these studies to evaluate structural and functional changes in eukaryotic cells in the first 24 hours following exposure to *C. rodentium*. Electrical transepithelial resistance of cell monolayers significantly decreased, while permeability of the cell monolayers to dextran (10 kDa and 40 kDa) increased after 3h incubation with *C. rodentium*. Confocal microscopy of the monolayers revealed that there was disorganization of tight-junctional proteins following 6-12 hours of bacterial exposure. Transmission electron microscopy showed bacterial attachment, pedestal formation, local disruption of tight junctions and an increased vacuolization. Our results suggest that the capability of *Ptk6* cells to differentiate into absorptive, goblet, and endocrine cells, and the characteristic changes they possess when exposed to *C. rodentium* make this cell line ideal for *in vitro* studies to define the cellular and molecular basis of its pathogenesis.

014P

Application of change-mediated antigen technology (CMAT) in the identification of *Francisella tularensis* gene products up-regulated during infection.

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Tularemia, caused by the facultative intracellular pathogen *Francisella tularensis*, is a potentially fatal disease in many hosts, including humans. Although the disease presentations have been reasonably well-characterized, the molecular mechanisms of pathogenesis of *F. tularensis* remain poorly understood. Our laboratory has previously employed a gene discovery methodology, known as *in vivo*-induced antigen technology (IVIAT), on *Yersinia pestis* and *Brucella abortus* to identify virulence-associated genes, up-regulated during infection. By this technique, we have been able to successfully identify numerous *in vivo*-induced genes among the two pathogenic species, whose products fall into common conserved functional categories. In this regard, we applied a variation of IVIAT methodology known as CMAT ("change-mediated antigen technology") to identify *F. tularensis* antigenic proteins up-regulated *in vivo*. Anti-serum was first generated by hyper-immunizing four New Zealand White rabbits with confirmed *F. tularensis*-positive reticuloendothelial tissues, inactivated for viable bacteria, from naturally infected animals. After assessing reactivity against *F. tularensis* whole cell lysates by Western blot, the serum was adsorbed multiple times with the *in vitro* grown pathogen to remove antibodies to constitutively expressed antigens. *Escherichia coli* expression libraries were constructed with *F. tularensis* genomic DNA, and probed with the adsorbed serum. To date, over 22,000 *E. coli* transformants have been screened, with nine sero-positive clones identified. While, sequencing results of the inserts revealed several

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putative gene products with metabolic functions, other clones carried *F. tularensis* sequences encoding open reading frames of potential virulence function, including a capsule biosynthesis protein, capB, a purine nucleoside phosphorylase, DeoD, and a conserved hypothetical membrane protein. Further analysis of the intact genes and the expressed recombinant products derived from these clones should provide insight into the biologic processes of *F. tularensis* uniquely associated with infection.

015P

Microbial protein-Antigenome Determination (MAD) technology: A proteomics-based strategy for rapid identification of microbial targets of host humoral immune responses.

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Purpose: Immunogenic, pathogen-specific proteins have excellent potential for development of novel management modalities. Here, we describe an innovative application of proteomics called Microbial protein-Antigenome Determination (MAD) Technology for rapid identification of native microbial proteins that elicit and interact with host humoral immune responses. Method: To demonstrate "proof of principle", we cultured the foodborne pathogen *E. coli* O157:H7 (O157), in vitro, in minimal medium supplemented with norepinephrine (NE), a β -adrenergic hormone found in the mammalian gastrointestinal tract, and a mimic of auto-inducer 3 that regulates expression of O157 proteins via quorum sensing. NE-induced O157 proteins were immunoaffinity captured using polyclonal antibodies from pooled hyperimmune sera of cattle, and defined by one dimensional SDS-PAGE gel liquid chromatography tandem mass spectrometry (GeLC-MS/MS), followed by SEQUEST database searching. Results: MAD identified 91 O157 proteins with genome wide distribution, as being part of the antigenome in bovine reservoirs including, previously identified O157 adhesins, O157 proteins identified using other proteomics-based approaches such as Proteomics-based Expression Library Screening (PELS; Kudva et al., Mol. Cell. Proteomics 5:1514-1519, 2006); and a subgroup O157 proteins expressed specifically during human infection (John et al., Infect. Immun., 73:2665-2679, 2005). Additionally, a subset of novel extracytoplasmic O157 proteins, unique to this study, was identified. Currently, the adherence potential of a number of these novel proteins is being systematically evaluated using cell assays, as a prelude to future animal experiments. Conclusion: The MAD technology is an innovative, rapid proteomics-based platform application for determining antigenomes expressed by microbial pathogens cultured in vitro under conditions that approximate the host environment. MAD can be applied to any sequenced pathogen that is capable of eliciting host humoral immune responses, and can complement other strategies for proteome-wide identification of immunogenic microbial proteins.

016P

Detecting differential protein expression between pathogenic and commensal *Staphylococcus aureus* using SILAC.

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Staphylococcus aureus is a leading causative agent of food-borne diseases, bovine mastitis and human skin infections. The production of staphylococcal enterotoxins (SE), antibiotics resistance and invasiveness makes this pathogen a serious concern in food and health industries. Commensal strains of *S. aureus* reside on the skin and in mucosal membranes of nearly 20-50% of animals and humans, however only a portion will become pathogenic. The differential expression of proteins in commensal and pathogenic strains is proposed here as the basis for variation in the rates for spread of infection. Twenty one commensal isolates were obtained from the bovine's hock, teat, and nose. Pathogenic strains were isolated from bovine cases of mastitis. Confirmation of strains as *S. aureus* was conducted using Polymerase Chain Reaction (PCR) with primers for *S. aureus* specific *vicK* gene. To determine optimal media for subsequent comparisons of protein expression using SILAC, bacterial growth curves were conducted. Growth of commensal and pathogenic *S. aureus* strains was compared in Dulbecco's mod[Unsupported Character - ﬁ]ed Eagle's medium (DMEM), Minimal Essential Media (MEM), Trypticase Soy Broth (TSB), Mannitol Salt Broth (MSB) and Roswell Park Memorial Institute (RPMI 1640). Only growth in DMEM did not differ significantly between commensal and pathogenic isolates over a 24hr period. Growing isolates in DMEM, containing light and heavy (^{13}C) isotopes of lysine will allow for identification of differentially expressed proteins using stable isotope labeling by amino acids in cell culture (SILAC). Combining these labeled proteomes, and generating peptide pools using few enrichment steps would enable quantification of protein levels using mass spectrometry. Final protein identification will be conducted using MASCOT software. The findings will serve not only as a model for potential vaccine development, but also in deciphering the evolution of the pathogenic bacteria and mechanisms through which it regulates the immune system. Keywords: *Staphylococcus aureus*, differential protein expression, Stable isotope labeling by amino acids in cell culture

017P

On the Trail of Regulatory T cells in Blood and Tissues of *Mycobacterium paratuberculosis* Infected Cattle.

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Based on previous observations, it has been proposed that induced regulatory T cells (Tregs) form an important aspect of immune responses to *Mycobacterium avium* subspecies *paratuberculosis* (MAP) in cattle. Tregs are typically CD4+CD25+ and express the FoxP3 transcription factor. Removal of CD25+ T cells from peripheral blood mononuclear cells (PBMCs) isolated from MAP infected cattle prior to stimulation with MAP antigens enhances production of IFN γ mRNA and reduces production of IL-10 mRNA from remaining cells. To more definitively identify antigen-specific Tregs in MAP infected cattle, we have begun to analyze cell populations in PBMCs using multi-color flow cytometry and intracellular staining. Tregs in tissues have been visualized using multi-color confocal microscopy and immunohistochemical staining. As expected, stimulation of PBMCs from infected cattle with MAP enhances the percent CD3+ cells that are also CD25+ (25%, +/-2.8%), relative to nil stimulated cells (6.4%, +/- 3.8%). A similar expansion of the CD25+ cell population is not observed in cells from MAP negative animals. Of note, stimulation of PBMCs with purified protein derivative (PPDj) did not drive significant increases in CD25 expression. Expression of the FoxP3 transcription factor is more variable between animals with some showing up regulation following stimulation with MAP (~20% of CD25+ cells) relative to nil stimulation (~6%). MAP stimulation of cells from test negative cattle may also enhance FoxP3 expression, suggesting this may be a general response to MAP antigens. Our data suggests that, in some cattle, CD25+FoxP3+ cells do indeed form a significant proportion of cells responding to MAP antigen stimulation. FoxP3 positive cells are also a significant feature in many lesions associated with MAP infection, although it is not yet known if these cells are antigen specific. We are now concentrating on demonstrating a functional role for MAP antigen responsive FoxP3+ T cells, on the further characterization of these cells both in vitro and in tissues, and on the ontological development of FoxP3 expressing regulatory T cells in MAP infected cattle.

BACTERIAL PATHOGENESIS POSTERS

018P

Highly pathogenic strains of *Salmonella* Enteritidis show enhanced tolerance to acid, oxidative stress and better survival in egg albumen.

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Purpose: *Salmonella* Enteritidis (SE) is a major cause of food-borne gastroenteritis in the USA with contaminated eggs being the major source of human infection. Nevertheless, not all poultry-associated SE strains are equally pathogenic. In a previous study, we identified SE strains with low- (LP) and high-pathogenic (HP) potential using a mouse infection model. The objective of the present study was to further examine the link between observed differences in pathogenicity and tolerance of these SE strains to adverse environments encountered in the chicken host.

Methods: The stationary phase cultures of HP (n=4) and LP (n=3) strains of SE were tested for their ability to survive in acidic environment similar to that of chicken stomach (pH 2.6), in oxidative stress (15 mM H₂O₂) that SE strains encounter within the chicken macrophages, and growth in egg albumen (pH 9).

Results: We found that the HP strains survived in significantly high numbers ($P < 0.05$) than LP strains in acidic environment and in the presence of 15 mM H₂O₂. All HP strains grew well in egg albumen whereas two LP strains did not survive in this condition. In addition, all HP strains were more invasive after oral inoculation in 3-day old chickens with high numbers of bacteria being recovered from spleen and liver. In contrast, none of the LP strains were found to be invasive.

Conclusions: These observations indicate that HP strains with enhanced tolerance to acid and oxidative stress may survive better in the acidic environment of chicken stomach and within chicken macrophages. In addition, differential survival of SE strains in egg albumen indicates that not all SE strains may have the ability to contaminate internal content of eggs. We hypothesize that differential transcriptional or translational regulation account for the observed differences in virulence and other phenotypes observed in this study.

019P

Evaluation of three real-time PCR assays for detection of pathogenic *Leptospira* species in canine urine samples

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Leptospirosis is caused by several pathogenic *Leptospira* species, and is an important infectious disease of dogs. In infected dogs, *Leptospira* can cause life-threatening renal, hepatic and/or hemolytic disease and can also be shed in urine. Early detection of infection is crucial for an effective antibiotic treatment of the disease. Microscopic agglutination test (MAT) is widely used for serological detection of leptospirosis. However, confounding test results are one of many caveats of MAT. PCR-based diagnostic tests offer a rapid means to detect pathogens with high sensitivity and specificity. Though different PCR tests have been developed for detection of pathogenic *Leptospira* spp., thorough evaluation of the assays' performance on dog urine samples has not been carried out. In this study, we compared the performance of 3 real-time PCR assays, one targeting the 16S rRNA gene and the other two targeting the *lipL32* gene. With DNA extracted from laboratory cultured pathogenic *Leptospira* spp., all 3 real-time assays showed 100% specificity and had identical lower limits of detection. Compared to a conventional, gel-based PCR assay, all three real-time PCR assays were 100-fold more sensitive. There was a 100% agreement among the 3 assays' results when tested on urine samples collected aseptically from dogs suspected for leptospirosis. However, when tested on urine samples that were collected by free-catch method, the 16S rRNA-targeting assay falsely detected 12% of the samples as positive for pathogenic *Leptospira* spp. Nucleotide sequence analysis of the amplified DNA fragments showed the assay resulted in false positives because of an unidentified bacterium. All urine samples collected from 100 apparently healthy dogs at a local animal shelter tested negative for pathogenic *Leptospira* spp. These results highlight the importance of sample-specific validation of PCR-based diagnostic assays and the application of appropriately validated assays for more reliable pathogen detection.

020P

Development of Cpb2 diagnostic ELISA and evaluation of the role of Cpb2 toxin in enteric disease in neonatal piglets.

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Purpose: Type A *Clostridium perfringens* has been identified as an important cause of enteric disease in piglets aged one to seven days, but most aspects of the disease are poorly understood. Several studies have shown the high prevalence and expression of the consensus *cpb2* gene in isolates from diarrheic piglets, which suggests that the Cpb2 toxin is a marker of virulence. The aim of the current study was to develop a sensitive diagnostic Cpb2 capture enzyme-linked immunosorbent assay (ELISA) for use in better understanding the role of Cpb2 in porcine neonatal diarrhea.

Methods: To analyze and specifically quantitate Cpb2, plates were coated with polyclonal antibodies (capture antibodies) prepared against recombinant consensus Cpb2 (rCpb2). After addition of consensus rCpb2 and small intestine and colon content samples, Cpb2 toxin was detected by a monoclonal antibody (Songer 9E4B) labelled with horseradish-peroxidase (HRP). To examine the correlation between the presence and the expression of the *cpb2* gene, piglet type A *C. perfringens* isolates were subjected to PCR, SDS-PAGE and Western blotting analysis.

Results: After optimisation of the capture ELISA (effects of plates, coating buffers, capture antibody concentrations, blocking agents, and detecting antibody concentrations), both recombinant and native consensus Cpb2 were detected in nanogram quantities, with a sensitivity down to 5 ng/ml and 50 ng/ml respectively. The optical density (OD) at 405 nm and 490 nm was protein concentration dependent over the range of 1-100 ng/ml. The assay is being tested using field samples from piglets with suspected type A *C. perfringens* infection and age-matched healthy control piglets. PCR, SDS-PAGE and Western blotting analysis showed that 13 of 14 isolates were positive for the presence of consensus *cpb2* gene and expression of consensus Cpb2.

Conclusions: These results support association between *cpb2*-positive type A *C. perfringens* and neonatal diarrhea in piglets.

BACTERIAL PATHOGENESIS POSTERS

021P

Sub-inhibitory concentrations of the antibiotic florfenicol reduces invasion in isolates of multi-drug resistant *Salmonella* Typhimurium DT104.

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Purpose: Sub-inhibitory concentrations of antibiotics can modulate global gene expression in bacteria and alter a wide variety of cellular processes, including motility, attachment, and invasion. For example, rifampicin inhibits transcription of shiga-like toxins in *Escherichia coli*, fluoroquinolones increase expression of colonization factors in *Clostridium difficile*, and florfenicol increases adherence of *Staphylococcus aureus*. To date, there has been little published research describing the effects of antibiotics on the virulence in multidrug-resistant *Salmonella*. Our research team investigated the impact of florfenicol on multidrug resistant *Salmonella enterica* serovar Typhimurium DT104 to determine whether alterations in the invasion rate of *Salmonella* DT104 occur during florfenicol exposure.

Methods: This was assessed genotypically through real-time PCR of genes associated with invasion and phenotypically through a cell culture invasion assay.

Results: Our results indicate that florfenicol decreases invasion of *Salmonella* DT104 at sub-inhibitory levels.

Conclusions: Sub-therapeutic levels of the antibiotic florfenicol do not enhance *Salmonella* DT104 virulence.

022P

Peptide nucleic acids inhibit *Brucella suis* in pure culture and infected macrophages.

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Purpose: There is an urgent need for alternatives to traditional antibiotics for the treatment of bacterial infections. The small number of novel antibiotics developed over the past 20 years combined with the misuse/application of current therapeutic regimens has led to increased frequencies of bacterial pathogen resistance to all classes of antibiotics. In both prokaryotic and eukaryotic cells, examples of antisense mechanisms have been found that lead to inhibition of transcription and translation, and in turn, inhibition of cell growth. One such example is peptide nucleic acids (PNAs). PNAs are short synthetic nucleic acid analogs (10-12 bases) in which the naturally occurring sugar-phosphate backbone has been replaced by a synthetic peptide. PNAs are long-lived, stable constructs that inhibit expression of targeted genes as well as growth of a number of bacterial species. However, the effectiveness of PNAs against intracellular bacterial pathogens, such as *Brucella*, has not been well characterized. Brucellosis in humans, principally caused by one of three *Brucella* spp., is one of the five most common bacterial zoonoses in the world. Moreover, due to the intracellular location of the brucellae, the disease is very difficult to treat with a relapse rate of up to 15%. The objective of this research was to assess the susceptibility of *B. suis* to a variety of PNAs in two different scenarios.

Methods: PNA constructs designed against several essential genes, as well as genes whose products are targets of current therapies. These constructs were then tested both in pure culture as well as in an infected murine macrophage model.

Results: The studies here show that PNAs targeted to *Brucella* genes encoding enzymes involved in DNA, RNA and protein synthesis inhibit the growth of *B. suis* in culture and in macrophages.

Conclusions: This study reveals the usefulness of PNA antisense constructs as novel therapeutic agents to *Brucella* infected macrophages and may portend their application to other intracellular pathogens such as *Salmonella* spp., *Francisella tularensis*, and *Mycobacterium tuberculosis*.

Key Words: peptide nucleic acid (PNA), *Brucella*, antibiotic resistance, mouse, macrophage

023P

Diversity of *Enterococcus cecorum* in chickens from Ontario

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Purpose: Little is known about *Enterococcus cecorum*, its ecology and significance in animals. It is part of the normal intestinal flora of chickens but has also been described as a cause of bone and joint lesions in broilers. *E. cecorum* infections have been increasingly reported in North America. In Ontario, *E. cecorum* infections were diagnosed for the first time in 2008 and *E. cecorum* is now regularly isolated from bone and joint lesions as well as blood filtering tissues of birds in broiler and broiler breeder farms across the province. The objective of this study was to assess if the increase in *E. cecorum* infections in Ontario was caused by the emergence of a particular strain and to determine the antimicrobial susceptibility profiles of field isolates.

Methods: *E. cecorum* was isolated from clinical infections in broilers and broiler breeders and from the ceca of healthy chickens using blood agar and Columbia agar with colistin and nalidixic acid (CNA), respectively. Identification was confirmed by biochemical tests and 16S rRNA gene sequencing. Isolates from a variety of farms were typed by pulsed-field gel electrophoresis (PFGE) and similarity between profiles assessed using Dice coefficients and UPGMA. Minimal inhibitory concentrations (MICs) were determined for 12 antimicrobial agents by broth microdilution and E-Test.

Results: PFGE demonstrated a large genetic diversity among isolates from healthy chickens, but the vast majority of clinical isolates belonged to a single cluster, independent of farm of origin. Higher MICs were generally observed in clinical isolates for macrolides, streptomycin, and possibly enrofloxacin, florfenicol, neomycin and gentamicin than in isolates from healthy birds. Lower clindamycin MICs seemed to be more frequent in clinical isolates.

Conclusions: The majority of clinical infections with *E. cecorum* in Ontario chickens appears to be caused by a single clonal lineage which may have emerged only recently in this province. This clone is associated with a particular antimicrobial resistance profile. Further investigations are needed to assess what makes this *E. cecorum* clone more successful as a pathogen than others.

BACTERIAL PATHOGENESIS POSTERS

024P

Problems of Epidemiological studies on Q fever in the Odessa region of Ukraine.

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The purpose of this study is to reveal the current state of Q fever, an important medical and social problem, based on etiological, clinical and epidemiological investigations in the Odessa region in 2008-2010. Results were analyzed to determine the incidence of Q-fever from different animal species in the Odessa region.

Sera from domestic and wild mammals (659) and from humans (314) were examined using the complement fixation test (CFT), enzyme-linked immunosorbent assay ELISA and indirect immuno-fluorescence. Ixodic ticks (8604) were identified, and then examined using immuno-fluorescent microscopy during the years 2008-2010.

The serological results indicated that the Kiliyskiy and Artsizsky areas were unfavorable for Q fever during 2008-2009, and in 2010, the Artsizsky, Bolgradsky and Tarutinsky areas were unfavorable for Q fever. The seropositive rate of the serum samples tested from animals in Artsizsky area during 2008 was 21.9%, in 2009 - 43.75%, and in 2010 - 66.7% were seropositive. In the Bolgradsky area in 2008-2009 there were no seropositive samples, and in 2010, 34% of the samples were seropositive. In the Tarutinsky area in 2010, 57.7% of the samples were seropositive.

In one of the Odessa districts, 21.9 % of the animals tested were seropositive in 2008, and in 2009, 43.75% were seropositive. In 2009, 21.95% of the human samples were seropositive and ticks, collected in this area, were positive for *Coxiella burnetti*. In 2010, no people were seropositive, but 66.7% of animal serum samples were seropositive, and ticks were positive, for *Coxiella burnetti*.

From the results of epidemiologic and epizootologic investigations, it was determined that there is a nidus of Q-fever in the Odessa area. The greatest amount of seropositivity in people is in the districts with the increased presence of seropositivity in domestic animals and *Coxiella burnetti* in ticks.

Epidemiologic and serological investigations showed the urgency of the problem of zoonotic disease in the Odessa region. Further systematical control and more modern laboratory investigations are required. Future investigations require new efforts and tight collaboration of both veterinary and medical services.

BIOSAFETY AND BIOSECURITY POSTERS

025P

Application of alternative methods of body temperature measurement in swine.

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Purpose: Increased body temperature can be seen with many swine pathogens including porcine reproductive and respiratory syndrome virus and influenza virus. Therefore, measurement of body temperature can be used to detect the presence of disease at the population or individual level and is often measured during infectious disease research studies. The objectives of this study were to assess the correlation between various methods of body temperature measurement in swine and to assess the validity of an infrared camera for the detection of elevated body temperature.

Methods: Thirty pigs were injected subcutaneously and intramuscularly with temperature sensing microchips at day 0 (Destron Fearing LifeChip® With Bio-Thermo® Technology). Pigs were housed at the University of MN Isolation Facility in groups of 10. A pig infected with influenza virus was introduced into each group at day 1. Body temperature was then measured for seven consecutive days by four methods: subcutaneous microchip (SQ), intramuscular microchip (IM), infrared camera (IR), and rectal thermometer (RT). Mean temperature measures and variability for each method were compared. Correlation and validity measures were also calculated.

Results: Rectal thermometer (RT) measures were the highest (104.3°F), followed by IM, SQ, and IR, respectively. Subcutaneous (SQ) and IR measures were both significantly lower compared to the RT and IM temperature measures. Rectal (RT) and IM measures were highly correlated ($r=0.86$). Infrared (IR) camera temperature measures were moderately correlated with IM measures ($r=0.61$). Infrared (IR) camera temperature measures had reasonable sensitivity (73%) and specificity (99%) values with elevated body temperatures.

Conclusions: The use of intramuscular microchips for temperature measurement eliminates the need to handle pigs, thus reducing stress on pigs and animal handlers. Therefore, monitoring body temperature via intramuscular microchips is ideal for animal research settings. The IR camera can likely be used effectively as a screening tool due to its reasonable sensitivity and specificity values with elevated body temperatures.

026P

Animal genetics rescue from diseased animals using somatic cell nuclear transfer (SCNT) technology

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The major obstacle for the animal agriculture industry is infectious disease. Disease outbreaks have caused severe economic losses worldwide. The unprecedented large-scale Porcine Reproductive and Respiratory Syndrome (PRRS) outbreaks in 2006 swept over nearly half of China's pig population involved more than 2,000,000 pigs, which posed great concern to the global swine industry and to public health. The recent foot and mouth disease outbreak in Japan has had more than 200,000 stock either destroyed or to be killed. The worst consequence of disease outbreak is the loss of the prized genetics. In this study, we evaluate the feasibility of using SCNT technology to rescue the genetics from diseased animals. Based on their prevalence and economic impact, Bovine Viral Diarrhea Virus (BVDV) in cattle and PRRSV in pig are selected as candidates for this study. Ear skin tissues from virus infected animals are collected and treated with disinfection agents and used to derive pathogen free fibroblast cells. The pathogen free cells are then used to produce animals with identical genetics of the diseased animal via SCNT technology.

EPIDEMIOLOGY AND ANIMAL HEALTH ECONOMICS POSTERS

027P

Associations of antimicrobial use and antimicrobial resistance in *Escherichia Coli* isolates individually sampled from feedlot cattle.

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Purpose: Current production systems for feedlot cattle utilize antimicrobial drugs (AMDs) for prevention and treatment of disease as well as for growth efficiency. Surveillance systems have been developed to monitor antimicrobial drug use (AMU) and antimicrobial resistance (AMR) in food animals due to concerns regarding public health. The objectives of this study were to document baseline AMR prevalence in feedlot cattle, and to investigate the associations between AMU with AMR in isolates of *E. coli* sampled from individual animals.

Methods: A two-stage random sampling plan was used to enroll 5913 cattle at four western Canadian feedlots. A rectal fecal sample was collected from each individual at arrival and during standard feedlot rehandling (at approximately 60 days on feed [DOF] or later). Parenteral and infeed AMU data were recorded for each individual enrolled in the study as well as for other animals in the same pen.

Results: For the second time point (>60DOF), a total of 2133 *E. coli* isolates from 1083 individuals in 251 pens were cultured and tested for susceptibility to antimicrobials by disk diffusion. At the >60DOF sampling point, adjusted resistance prevalence was 25.6% (95%CI: 23.5-28.0) for sulfonamide, 25.0% (95%CI: 22.8-27.3) for streptomycin, and 72.7% (95%CI: 70.5-75.1) for tetracycline. Arrival adjusted resistance prevalence for sulfonamide was 7.5% (95%CI: 6.1-9.2), for streptomycin was 7.7% (95%CI: 6.3-9.5) and for tetracycline was 20.0% (95%CI: 17.7-22.6). All other AMD tested had AMR prevalences less than 10% at both sampling points.

Conclusions: Multivariable logistic and linear modeling with nested effects for individuals and pens identified weak associations of tetracycline and macrolide AMU classes with various resistances and no association with other AMD classes.

028P

Isolation and characterization of methicillin resistant *Staphylococcus aureus* from bulk tank milk in Minnesota dairy farms.

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Staphylococcus aureus, is a major cause of mastitis in dairy cattle and is frequently associated with antibiotic use on farms. The current study was undertaken to determine the prevalence of methicillin resistant *S. aureus* (MRSA) in dairy farms around Minnesota. Farm prevalence of *S. aureus*, including MRSA, was estimated from bulk tank milk (BTM) samples and isolates were characterized genotypically and phenotypically. A total of 150 pooled BTM samples from 50 farms spanning the 3 seasons (spring, summer and fall of 2009) were collected. The prevalence of any *S. aureus* was: 62% while that of MRSA was: 5.3%. All 101 isolates were subjected to antibiotic susceptibility testing using the Kirby Bauer method. Of these 54 were pansusceptible, 2 were resistant to a single antibiotic, 37 MSSA were resistant to >2 antibiotics. All the 8 MRSA isolates were multidrug resistant. Staphylococcal protein A or *spa* typing identified that *spa* types t529 and t034 were most prevalent among MSSA and types t034 and t359 among MRSA. All MRSA isolates were also genotyped using MLST and PFGE profiling. Of the 8 MRSA, one isolate had a composite profile of - ST 5-USA 100-*spa* 2 type which has been reported among HA MRSA, while a second carried - ST 8-USA 300- t121 type commonly identified amongst CA-MRSAs. The third had: ST 398- untypable by PFGE-t034, reported for LA-MRSA particularly of porcine origin. Four other isolates carried genotype combinations similar to those reported for CA-MRSA. This suggests that MRSA associated with hospitals, community and livestock can all be found in dairy farms. Large-scale studies on MRSA are needed to understand factors associated with their emergence and persistence in dairy environments.

029P

Molecular characteristics of Ukrainian Laboratory and industrial strains of *Bacillus Anthracis*.

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Purpose: Although some genetic heterogeneity is evident in commercial and laboratory strains, *Bacillus anthracis* is one of the most monomorphic microbes among known bacterial species - a fact which complicates genetic strain identification. Inadvertant reintroduction of pXO2 plasmid into a vaccine strain can have dire consequences. This study aimed to develop and use multiplex PCR to genetically characterize Ukrainian laboratory and industrial *B. anthracis* strains (both capsular and acapsular).

Methods: Using developed multiplex PCR, we performed molecular characterization of *B. anthracis* strains maintained in Ukraine - K79Z, 55, 34F2, SB-072, Stea (Ukraine), Sterne (Ukraine), Sterne (Georgia), and Tsenkovskogo-2 (M71; Ukraine). Strain characterization included detection and analysis of pXO1 (PA gene promoter, fragments, and complete PA gene) and pXO2 (capC; analysis of mutations in the *atxA* gene) plasmid sequence variations; and analysis of chromosomal loci for the *Sap* gene, Ba813, and VNTR (*vrnA*, *vrnC1*, *vrnC2*, CG3).

Results: All aforementioned strains exhibited identical PCR amplification patterns except for Tsenkovskogo-2 (a capsule-containing strain possessing both plasmids), which exhibited a shorter *vrnC2* locus in VNTR.

Conclusions: These data suggest that VNTR is a useful marker to identify the presence of Tsenkovskogo-2 among the commonly used *B. anthracis* vaccine strains in Ukraine. PCR amplification of VNTR represents a source of molecular variation in *B. anthracis*, and we suggest that VNTR may be useful for characterizing new *B. anthracis* isolates. Locus *vrnC2* in VNTR may also serve as a molecular marker to control for contamination by Tsenkovskogo-2 during production of anthrax vaccines in Ukraine.

030P

Direct and indirect contact rates among Vermont dairy farms.

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Purpose: Contact rates are required to model disease spread in populations of animals or farms. To characterize direct and indirect contact rates among dairy herds in Vermont, a mail survey was conducted in spring 2010.

Methods: Surveys were mailed to one-half of the dairy farms inspected by the Vermont Agency of Agriculture. Every other farm on a list sorted by county was selected to participate. Of 500 surveys mailed, 268 were returned with usable responses. Information on recent animal movements, farm visitors, and biosecurity practices was reported.

Results: Preliminary analysis indicates 23% of farms purchased cattle in 2009, with over 56% of purchases being from known sources. Twenty-six percent of farms were visited by a milk truck at least daily. Most farms (71.5%) indicated every other day milk pick up. Almost one-third reported never using the service of an artificial breeding technician; just over one-third reported never hiring a hoof trimmer; and about one-quarter were visited by a veterinarian less than once a month. About 50% were visited by an artificial breeding technician at least once a week; about 50% were visited by a hoof trimmer less

EPIDEMIOLOGY AND ANIMAL HEALTH ECONOMICS POSTERS

030P (continued)

than once a month; and 74% were visited by a veterinarian more than once a month.

Conclusions: These rates of animal movements and indirect contacts will be useful as inputs for a model such as the North American Animal Disease Simulation Model to assist in developing response plans to foot-and-mouth disease or other highly contagious diseases.

031P

Vaccination of calves up to 15 months of age against *Mycobacterium avium* ssp. *paratuberculosis* impacts Johne's disease incidence and cull rates.

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Johne's disease (JD) caused by *Mycobacterium avium* ssp. *paratuberculosis* (MAP) is significant and costly to dairy producers. Vaccination with USDA approved vaccine (Mycopar) between 5 and 35 days of age reduces the incidence of clinical disease by 89-93%. One major limitation of vaccination with Mycopar is economic losses incurred during the period between vaccination and integration into the lactating dairy herd. This pragmatic trial examined the efficacy of Mycopar vaccination in dairy heifers up to 15 months of age on the incidence of Johne's disease and shedding of MAP. Two hundred fifty six heifers aged 5 days to 15 months from one dairy herd with a known MAP prevalence of greater than 10%, were blocked by age and enrolled in this trial. The diagnosis of MAP infection in cattle enrolled in the study was based on MAP culture of fecal samples collected at 42 months of age. Fecal culture results were analyzed with a Fisher's exact test. Cull rates, MAP DTH responses, and vaccine reactions were analyzed using an ANOVA. The total number of culls due to clinical MAP infection was reduced from 25 per year prior to the introduction of vaccinated animals to 8 animals in the fourth year of the study ($p=0.024$). There was no significant increase in the number or size of adverse vaccination site reactions. While not statistically significant, the incidence of MAP infection quantified by fecal culture was reduced after 42 months ($p=0.14$). This study suggests that juvenile vaccination is effective in significantly reducing the number of animals with clinical Johne's disease, and should be further evaluated as a means to improve the economics of Johne's disease management.

032P

First report of the bovine lymphotropic herpesvirus (BLHV) within Canada and detection of the viral genome in tissues of a bovine aborted fetus.

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In late 2008, an epidemiological survey in Québec, Canada, was initiated to investigate the pathogens involved in cow abortion. Thus, several PCR-based assays were done on placenta and tissues of bovine aborted fetuses for the detection of bovine pathogens that are known to be involved in abortion. From 26 submitted cases tested of bovine abortion, none were positive for BVDV and *Leptospira* spp., whereas 3.8%, 11.5%, and 3.8% were positive for IBR, *N. caninum*, and *C. burnetii*, respectively. A pan-herpesvirus nested-PCR assay was also conducted on placenta and pooled tissues of aborted fetuses. Interestingly, a pan-herpesvirus PCR positive result (FMV09-1125585; GenBank accession number HM152484) was sequenced and comparison by BLAST showed 100% identity with a 483 nucleotides fragment of the DPOL gene of a previously reported BLHV (GenBank accession number AF327830), which is a virus classified in the *Herpesviridae* family within the subfamily *Gammaherpesvirinae*. To our knowledge, this is the first time that BLHV has been found in the bovine species in Canada. BLHV has been previously reported only in United States (in 1998) and United Kingdom (in 2006). Since the first assay on a pool of tissues of the aborted fetus gave a negative result and to further investigate the case, individual tissues were tested by nested-PCR and amplicons that were produced were sequenced. Interestingly, brain and lymph nodes were BLHV positives and, to our knowledge, this is the first time that BLHV was detected in tissues of a bovine aborted fetus. Samples of spleen, liver, kidney, and heart were BLHV negative. No histopathological lesion suggestive of a viral infection could be found in BLHV nested-PCR positive tissues. Until now, the involvement of BLHV in the induction of any diseases is still uncertain. Moreover, the presence of *N. caninum* was confirmed by PCR in the BLHV positive case. Nonetheless, it is important to report the presence of BLHV because this is the first step toward the improvement of bovine pathogen surveillance.

033P

Factors associated with bovine liver condemnations in Ontario Provincial Abattoirs 2001-2007.

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Purpose: Portion condemnation data may play an important role in a food animal syndromic surveillance system, as these data may provide more specific results than whole carcass condemnation data. Various seasonal, secular, disease and non-disease factors have been previously identified to influence whole carcass condemnation rates in Ontario provincial abattoirs, and if ignored, may bias the results of quantitative disease surveillance methods. The objective of this study was to identify variables which may impact liver condemnation rates and compare how these variables may differ from factors associated with bovine whole carcass condemnation rates.

Methods: Data were collected from the Ontario Ministry of Agriculture, Food and Rural Affairs (OMAFRA) and the Ontario Cattlemen's Association regarding parasitic liver portion condemnation rates for different cattle classes, abattoir compliance ratings, and the monthly sales-yard price for commodity classes from 2001-2007. To control for clustering by abattoirs, multi-level Poisson models were constructed to examine the association between the following variables and parasitic liver condemnation rates: year, season, annual abattoir audit rating, geographic region, number of weeks per year an abattoir was operating, total number of animals processed each year, animal class and commodity sales price.

Results: Results showed that parasitic liver condemnation rates appeared to be associated with year, season, animal class, audit rating, and region. Unlike previous models based on whole carcass condemnations, commodity price did not appear to be a significant variable for liver condemnations.

Conclusions: These results suggest that different variables may be associated with condemnation rates depending on the type of material being condemned, and should be modeled and controlled for during quantitative surveillance analysis on a portion specific basis.

034P

Multiple introductions of North American type 2 porcine reproductive and respiratory syndrome viruses into Thailand.

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Porcine reproductive and respiratory syndrome virus (PRRSV) is a major swine virus causing heavy economic losses in the swine industry worldwide including Thailand. Porcine reproductive and respiratory syndrome (PRRS) was first evident in North America in 1987 and later in Europe in 1990. PRRSV has undergone rapid expansion in both genetic diversity and population size since its first discovery in the mid 1980s. Its rapid evolution is often followed by extensive and distance-independent geographical dispersion which amplifies the influence of some regional viral strains and causes regional and nation-wide prevalence of lethal disease. In Thailand, PRRSV was first isolated in 1996 but serologically evident since 1989. Our previous report showed that both EU (Type 1) and NA (Type 2) genotypes exist in Thailand and sequential analysis of ORF5 gene confirmed genetic variation of Thai PRRSV. During the 2001 PRRS outbreaks in Thailand, the PRRS virus 01NP1 was isolated from intensive swine farming areas. Additional 6 NA type viruses isolated in Thailand from 2007 to 2008 were also included in this study. According to the lineage classification of NA (Type 2) PRRSV published by Mang Shi et. al. (2010), the NA type isolates of Thailand were located in 2 different lineages such as lineage 1 and 6. This finding indicates the NA type PRRS outbreaks in Thailand were caused by multiple introduction of NA type PRRSV. Since its first appearance in Thailand, PRRSV has been the major cause of economic losses in the swine industry. Until now, Thai authority does not allow any use of modified live virus vaccine of PRRSV, and only the killed virus vaccine is commercially available since 1996. Therefore, the importation of swine breeder from North America represents one of the possible reasons for Type 2 PRRSV introductions into Thailand.

035P

A web-based database and phylogenetic tools to study molecular epidemiology and evolution of Porcine Reproductive and Respiratory Syndrome Virus (PRRSV).

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Purpose: Our goal is to develop a web tool - PRRSV-Webtool, which stores Porcine Reproductive and Respiratory Syndrome virus (PRRSV) genome sequences and related data, to allow the users performing analysis easily via internet.

Methods: PRRSV-Webtool integrates three important components: database, phylogenetic tools and online resources. It stores PRRSV genome data and other data about PRRSV associated disease, which are updated constantly. Moreover, different phylogenetic tools are integrated into PRRSV-Webtool to allow the users to understand the epidemiological and evolutionary characteristics of their PRRSV isolates.

Results: We introduced an integrated and user-friendly web platform for the analysis of PRRSV genome data - PRRSV-Webtool. Users can study their own PRRSV sequences easily over the internet. Utilities in PRRSV-Webtool allow the users to understand how their PRRSV isolates genetically relate to other existing PRRSV strains, and other in-depth information about the virus evolution, such as the existence of recombination and time of divergence.

Moreover, studies in Europe and China both show that geographical location is an important factor influencing the molecular evolution of PRRSV. PRRSV-Webtool also provides a mapping function which helps to understand the geographical dispersion of the disease and the order of transmission of the virus.

Conclusions: With our PRRSV-Webtool, users (including virologists, veterinarians and porcine epidemiologists) can improve their understanding of PRRSV and hopefully move towards a better of control this virus.

036P

Case study of two vector-borne diseases in humans and animals of the far North region of Cameroon: Implications for preventative measures.

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Purpose: Vector-borne diseases, such as trypanosomiasis and malaria, are causes of significant economic losses to livestock production and human productivity in the developing world. However, disease control programs have met with limited buy-in from local populations.

Methods: In order to understand the way pastoralists think about vector-borne diseases and their prevention, we conducted 35 semi-structured interviews with FulBe mobile pastoralists of the Far North region of Cameroon.

Results: After comparing perceptions on disease prevention, we found that pastoralists are more likely to take an active role in preventing a vector-borne disease in cattle rather than in human populations. As well, mobile pastoralists perceive animal diseases as separate and different from human disease; animal diseases are described based on internal and objective findings whereas human diseases are typified by feelings.

Conclusions: This difference in perception suggests that, when working in an international public health setting, health care providers should recognize how economic and cultural significance can affect the use of preventative medicine resources.

037P

Situation of Rabies in Thailand, 2009.

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Thailand was located in South East Asia, throughout country had laboratories rabies compose of laboratory of DLD and Ministry of Public Health (MOPH), University, Thai Red Cross society. Almost these lab use Fluorescent Antibody technique (FA) and follow by inoculation into mouse's brain for confirm. Those will accept brain or head of animal from office where closed area such as provincial health office, provincial livestock office, private clinic or general people who would like to send samples by free of charge. From 2001 - 2008 had human rabies dead were cases 181 people. Animal rabies cases were dramatically decreasing from 46.62 % to 18.10 % during 1998-2008. Situation of rabies cases can found in all regions. We conduct retrospective study for describe rabies situation, determine magnitude of problem, distribution and recommend prevention and control measures by reviewed R506 surveillance data of Bureau of Epidemiology, Ministry of Public Health for rabies human cases that found 24 cases in 2009 and laboratory surveillance data at BADC that collect rabies testing data from laboratory all country between 2009. We found rabies positive 29.02% (341/1,175) submitted samples, 33.43% (114) found in Bangkok and other found distribution throughout Thailand. Highest incidence per 100,000 of dogs in Songkla province 89(25 samples) Patumthani 67(39 samples), Mukdahan 64 (5 samples) Nong Bua Lum Pu 56 (5 samples) and Bangkok 42 (114 samples) From highly incidence of animal rabies cases in Thailand, almost that were coincide with human cases. Authorities organization should aware incidence cases in human, pay attention in vaccine coverage in both owner and stray dogs and cats, preparing rabies antiserum for affected people from animal contact or bitten. Should seek new control measure in endemic area like Bangkok such as Oral Rabies Vaccine. Implement surveillance system for animal rabies detection

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and collaborate in share laboratory information between human and animal sides. Strengthen to use animal registry laws, Animal Epidemic laws. Control stray dog and cat population by sterilization and establish habitation for them for decreasing incidence of rabies cases.

038P

A mathematical model to predict the effect of antimicrobials on the efficiency of horizontal gene transfer.

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The widespread use of antimicrobials in medicine as well as in food production has resulted in pathogenic bacteria becoming resistant to the antimicrobials used to treat them. Antimicrobial resistance (AMR) can result from horizontal gene transfer (HGT) through plasmids and transposons. The exact influence of the antimicrobial concentration on HGT is, however, not clearly defined. The objective of our study was to develop a mathematical model using in vitro data to characterize the influence of the antimicrobial oxytetracycline on the rate of conjugative transfer of genetic material between enteric pathogens. A clinical isolate of *Salmonella* Typhimurium containing a 100kb conjugative plasmid that encodes resistance to ampicillin, chloramphenicol, streptomycin, sulfisoxazole, tetracycline, kanamycin, ceftriaxone, and cefoxitin (Giles et al., 2004) was used as donor and a laboratory strain of *E. coli* (C600N) as recipient. Broth mating was performed in the presence of increasing concentrations of oxytetracycline and compared to antimicrobial-free medium. A previously described mathematical model (Gehring, et al., 2010) was fit to these data and the horizontal transfer efficiencies of a multidrug resistant conjugative plasmid from *Salmonella* to *E. coli* were determined. The in vitro data showed highly similar patterns of HGT efficiencies when compared to the predicted efficiencies. This study provides an important tool in defining the role of antimicrobial concentration in HGT and may provide future insight into better control of AMR.

039P

Monitoring of Viral Haemorrhagic Septicaemia of Rainbow Trout in Ukraine.

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Purpose: Viral hemorrhagic septicaemia (VHS) of rainbow trout is a highly contagious disease, afflicting more than 45 species of sea and river fish. VHS is present throughout most countries of the world, including Western Europe. As the presence of VHS in Ukraine has not been previously described, the current study aimed to monitor VHS in Ukraine and to isolate VHS viruses possibly circulating within the country.

Methods: Reverse transcription (RT)-PCR and Ab-ELISA methods were used to detect VHS in rainbow trout. RT-PCR was performed using primers recommended by OIE and a test-system developed in Ukraine on the basis of "wobble-position" primers specific for nucleoprotein and glycoprotein, taking into consideration variations in VHS genotypes. Virus isolation was carried out using cell line BF-2. Reference strain "DH-4p101" (genotype 1a, Denmark) and two isolates, "PL1" and "PL2" (Poland), were used as positive controls.

Results: Rainbow trout biomaterials were sampled in all of the registered farms, which are located in 7 regions of Ukraine - 30 fishes from each farm in late autumn and early spring when the temperature was optimal for virus reproduction - 2-4°C. Special attention was paid to the regions neighboring Poland and Turkey, countries where VHS occurs. Studies were performed both with pooled whole fish (10 fishes) and with separate individual fish organs. VHS was not detected neither by "blunt" passaging in cell culture nor by RT-PCR. In addition, we failed to observe VHS-specific antibodies in any of our experimental samples.

Conclusions: Based on our sampling in seven Ukrainian regions, our data suggest that during the timeframe of 2009-2010, there was no evidence of VHS infection of rainbow trout.

040P

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

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Purpose: 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.

Methods: Nasal swabs collected daily from 16 of 21 horses affected with EHM were evaluated by a previously reported quantitative polymerase chain reaction technique.

Results: This outbreak involved three facilities linked by a common exposure, a riding clinic. In total, 21 of 111 horses naturally exposed to EHV-1 developed clinical signs of EHM. Of the horses tested, the last day EHV-1 nasal shedding was detected was Disease Day 9 with 6 of 13 shedding until Disease Day 6. EHV-1 was detected in nasal secretions of 43.8% of horses tested on Disease Days 0 to 3. There was high variability with nasal shedding and severity of neurologic signs.

Conclusions: Based on these findings, in the absence of laboratory testing, we recommend biosecurity measures be utilized when managing EHM cases a minimum of 9 days beyond EHM onset. Additionally, this report illustrates the potential for EHV-1 to spread between farms and within a large animal referral hospital reinforcing the need for biosecurity measures to be a normal part of daily operations at equine boarding and medical facilities.

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041P

Cluster analysis of methicillin resistant *Staphylococci* isolated from dogs and cats in Iowa.

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Purpose: Reports of zoonotic and nosocomial outbreaks of methicillin resistant *Staphylococcus aureus* (MRSA) infections in veterinary hospitals affecting humans and animals have increased in the last several years. Detection of an outbreak frequently relies on the actions of observant individuals within a particular veterinary facility. However, these outbreaks tend to be reported only within the affected hospital and viewed in a community-wide perspective. Therefore clusters of spatially-related MRSA or other methicillin-resistant *Staphylococcus* (MRS) isolated from pets may not be recognized due to a lack of central analysis. The veterinary diagnostic laboratory can serve as source of population-wide information to perform such an analysis. **Objective:** The objective of this study was to investigate the use of spatial analysis in detecting clusters of MRS cases from dogs and cats diagnosed at ISU VDL over a 7 year period. **Methods:** Information from canine and feline cases where *Staphylococcus* species was isolated, antimicrobial susceptibility testing performed and location information available during the 2003-2009 period was retrieved using the laboratory's information management system (LIMS). Location information was restricted to Iowa counties. Cluster analysis was performed using a spatial analysis program with Poisson- and Bernoulli-based models. **Results:** Seven hundred and fifty one (751) cases with the required information were retrieved from LIMS. Of these, 104 cases of MRS were found in Iowa, including 24 MRSA cases. Spatial analysis identified primary clusters by either Poisson or Bernoulli models with probability values (p-values) ranging from 0.004-0.225 for MRS and 0.108-0.210 for MRSA. **Discussion:** This study demonstrates that clusters of methicillin-resistant *Staphylococcus* from companion animals may be identified using diagnostic data and an easy to use spatial analysis program. Further investigation of the clusters may lead to sources of bacterial spread. Additionally, knowledge of potential clusters of MRSA within a community may alert veterinarians or physicians to investigate suspected cases more frequently or thoroughly.

FOOD AND ENVIRONMENTAL SAFETY POSTERS

042P

Bulk tank bacteria and selected foodborne pathogens in raw milk of Wisconsin farmstead dairy producers.

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Purpose: To assess the prevalence of bacterial population and selected foodborne pathogens in bulk tank milk produced on 9 WI farmstead dairies.

Methods: Initial enrollment of farms began on March 2010. Trained farm staff was asked to collect bulk tank milk samples for 15 consecutive weeks.

Samples were frozen at the farm and picked up monthly. Milk was used to perform standard bulk tank culture, total coliform count and laboratory pasteurized count. Culture for *Listeria* and *Salmonella* were done using enrichment techniques. Additionally, one fresh bulk tank milk sample was collected per farm to detect the presence of *Campylobacter* and *Mycoplasma*.

Results: Farms were located in distinct regions of WI, had a wide range of size (13 to 950 lactating cows), different management styles (confined and grazing) and produced a variety of dairy products (pasteurized and unpasteurized cheese, fluid milk, ice cream). A total of 107 weekly bulk tank samples were collected. *Staphylococcus aureus* were recovered for 36 weeks (prevalence of 33.6%) at least once in 8 of the 9 farms. *Streptococcus agalactiae* were recovered for 4 weeks (prevalence of 3.7%) from milk obtained from 2 different farms. Counts were 0 to 25,000 cfu/mL and 0 to 58,000 cfu/mL for Coagulase Negative *Staphylococcus* and Environmental *Streptococcus*, respectively. Bacterial populations were quite constant within farm showing counting variations within weeks per farm. Total coliform count was 446 and ranged from 15 to 15,000 cfu/mL. The laboratory pasteurized count was 36 and ranged from 25 to 120 cfu/mL. Of coliform counts, 22% were greater than 100 cfu/mL. *Listeria monocytogenes* were recovered for 3 weeks, 9, 12 and 13, (prevalence of 2.8%) from a single farm. *Listeria innocua*, a non-pathogenic specie of *Listeria*, were recovered twice from 2 different farms. *Salmonella typhimurium* was detected once (prevalence of 0.9%) from a farm with a history of salmonellosis in cattle. *Campylobacter* and *Mycoplasma* were not recovered but sample size was limited because a single fresh milk sample was used.

Conclusions: Results demonstrate bacterial quality of raw milk produced by WI farmstead dairies is similar to dairy farms producing for off-site processing.

043P

Factors associated with coliform count in unpasteurized milk

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Purpose: The objective was to identify factors associated with coliform counts (CC) in bulk milk.

Methods: Ten dairies were visited on alternate weekdays over a period of 10 weeks. For each visit, in-line drip samplers were used to collect milk samples from 2 points of the milk line (between the receiver jar and milk filters, and after the plate cooler). While in-line milk samples were collected, students observed milking hygiene and collected liner and teat skin swabs. CC was determined for milk and swabs using Petrifilm CC plates. CC was also performed at the dairy plants' lab using milk from the same tanker (tanker CC) that was loaded while in-line samples were collected. A mixed model was used to assess the association between in-line milk coliform count (ILCC) and a number of potential predictors.

Results: Median herd size was 1,227 cows and the mean duration of each visit was 73 minutes. Of 181 milk samples, ILCC was 37.3 CFU/mL and varied by farm (range = 4.6 to 1,198 CFU/mL. ILCC was 4 times greater (114.7 CFU/mL) when wash failures occurred (failure to reach pre-set wash water temperature (< 20%), dispense normal amount of detergent or skipped wash), as compared to ILCC after normal washes (26.4 CFU/mL). Pre-filter and post-cooler ILCC were not different for early (< 33% of the herd milked) or mid milking (33 to 66 %) whereas post-cooler ILCC was greater than pre-filter at late milking (67 to 100% of the herd milked) ($P < 0.01$). Mean ILCC (CFU/mL) increased 6.3% for every 10% increase in in-line milk SCC (cells/mL) and increased 2.3% for every 10% increase in liner CC (CFU/mL). Of 48 paired CC (tanker and in-line samples taken on the same day), tanker CC was greater than ILCC but the difference decreased from 154.8 at early milking, 62.6 at mid-milking to 1.0 CFU/mL at late milking ($P < 0.01$).

Conclusions: Results suggest that milking machine factors greatly influenced CC in bulk milk. CC determined at the plant intake was greater than CC prior to loading. The greater difference between ILCC and tanker CC at early milking may be a result of coliform growth during long tanker loading periods.

044P

Methicillin resistant *Staphylococci* found in clinical mastitis samples from dairy cattle in Iowa.

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Purpose: *Staphylococcus aureus* (SA) is a major cause of mastitis in dairy cows responsible for substantial economic losses in these industries.

Additionally methicillin resistant *Staphylococcus aureus* (MRSA) is a global health concern and has been isolated in foods of animal origin. Many MRSA strains also have increased resistance to tetracyclines, aminoglycosides, macrolides, lincosamides as well as β -lactam antimicrobials. Objective: The objective of this study was to investigate bovine milk samples for the presence of MRSA and determine the antimicrobial susceptibility profiles of the recovered isolates. Methods: *Staphylococcus* isolates were recovered from milk samples submitted to ISU VDL during June to October, 2009 time period. The milk samples came from animals with clinical mastitis. Bacterial identification was accomplished by evaluation of colony morphology and hemolytic pattern (single zone, wide zone Beta, or double zone) and biochemical tests (coagulase, maltose, lactose, trehalose, Voges-Proskauer). Methicillin-resistance was determined by screening isolates for sensitivity to oxacillin and activity of *mecA* with PBP 2a latex agglutination test. Antimicrobial susceptibility testing was accomplished by broth dilution using minimum inhibitory concentration (MIC) method. Results: Two hundred and one (201) staphylococcal isolates were identified from the milk samples. Of these, 62 were coagulase positive (CPS) and 139 coagulase negative (CNS). Oxacillin resistance was determined in 20 CNS and 7 CPS. Of the seven oxacillin resistant CPS, 6 were determined to be SA and only one of these was positive by PBP 2a test. Antimicrobial susceptibility profile for the MRSA isolate was similar to that found with the oxacillin resistant SA isolates. Discussion: These findings suggest that MRSA is not a common organism isolated from dairy cattle with mastitis. Less than 0.05% of the *Staphylococci* isolated in this study were identified as MRSA. Since *S. aureus* is a common organism in bovine mastitis, continued surveillance is needed to detect changes in prevalence which could become problematic.

045P

Acute host stress increases horizontally mobilizable plasmids and antimicrobial resistance genes in cattle feces.

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The incidence of antimicrobial resistance (AMR) among pathogenic bacteria is increasing. The influence of host environmental factors on this phenomenon is poorly understood. Among bacteria, conjugation is the most prevalent and efficient mode of horizontal transfer of genes, including those

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that encode virulence and antimicrobial resistance. We have previously reported that norepinephrine, a catecholamine released during acute host stress, enhances bacterial conjugation *in vitro*. In the present study, microbiome of fecal samples collected from cattle experiencing acute distress associated with dehorning and castration without anesthesia were compared to that of control animals. An initial diagnostic microarray revealed an increase above baseline in IncQ *oriV* in feces following surgeries. Quantitative PCR assay confirmed that the number of copies of *oriV* increased significantly following surgery (p value=0.0277). The copy number of sulfonamide resistance gene (*sulII*) that has been reported to be carried by IncQ family of mobilizable plasmids also increased following surgery (p value = 0.0017). Pathogenic bacteria have co-evolved with their hosts and have developed pathways to sense host health and sickness to modulate their own growth and survival. This study suggests that acute host distress may influence the transfer of antimicrobial resistance elements between bacteria.

046P

Transcriptional response of *Campylobacter jejuni* to erythromycin exposure.

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Purpose: *Campylobacter jejuni* is a frequent cause of foodborne illness in industrialized countries. Erythromycin is the drug of choice for treatment of campylobacteriosis, but resistance to this antibiotic is rising in *Campylobacter*. How *C. jejuni* adapts to erythromycin treatment is unknown. The aim of this study is to determine the molecular basis underlying *Campylobacter* response to erythromycin treatment.

Methods: The transcriptomes of *C. jejuni* 11168 treated with a lethal (16x MIC) or sub-lethal (0.5x MIC) dose of erythromycin were compared with that of the non-treated cells by competitive microarray hybridizations. Representative genes identified to be differentially expressed by the microarray were further confirmed by semi-quantitative real-time PCR.

Results: For the treatment with a lethal dose, a total of 258 genes were differentially expressed ± 2 -fold, $P < 0.01$, $Q < 0.05$ when compared to the non-treated culture. Among these, 139 genes were up-regulated and 119 were down-regulated. Many of the up-regulated genes are involved in flagellar biosynthesis and motility, amino acid biosynthesis, membrane functions, signal transduction, and multidrug resistance. The down-regulated genes are mainly involved in tricarboxylic acid cycle, electron transport, ATP synthesis, ribonucleotide biosynthesis, capsular biosynthesis, and C4-dicarboxylate utilization. For the treatment with a sub-lethal dose, fewer genes were differentially expressed, but many of the affected functional categories are consistent with those of the lethal treatment.

Conclusions: These findings suggest that in response to erythromycin treatment, *Campylobacter* increases motility and membrane protein expression (including drug efflux transporters) and decreases energy metabolism. These changes may facilitate *Campylobacter* to survive the treatment and develop antibiotic resistance.

047P

Comparative studies of heavy metal concentration in Prawn and Water samples from Epe Lagoon and Asejire Rivers in Nigeria.

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Whole prawn specimens and water samples from Epe Lagoon, in Lagos State and Asejire River, in Oyo State of Nigeria were analysed quantitatively for the presence of manganese, copper, zinc, lead and cadmium using Techcomp AA 6000 atomic absorption spectrophotometer. The mean concentration of each heavy metal in prawn specimens and water samples from both locations and the bioconcentration factor were determined. The results showed that the mean concentration of all the metals in prawn specimens were higher in Asejire River than in Epe Lagoon except for Lead. Cadmium had the lowest mean concentration in both locations. The mean concentration of metals in prawn specimens from both locations were all above the maximum limit recommended by WHO and FAO except for Zinc. The mean concentration of the metals in water samples from both locations were within the limits recommended by WHO and FEPA.

The results show bioconcentration and the contamination of the water bodies and prawn with heavy metals, which portend a serious public health risk. Consequently, continuous environmental pollution monitoring to check heavy metal hazards is hereby recommended.

048P

Antimicrobial resistance and resistance genes in *E. coli* and *Salmonella* isolated from salmon and shrimp purchased in Canada.

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Aquaculture is growing worldwide and antimicrobials are used for treatment and prevention of disease in aquacultured species. Regulations and use practices differ among producing countries. The potential for antimicrobial resistance and transmissible resistance genes to emerge in the zoonotic and reservoir bacteria of aquacultured species is a public health concern. The Canadian Integrated Program for Antimicrobial Resistance Surveillance (CIPARS) monitors the prevalence and resistance of enteric bacteria in beef, chicken and pork meat at retail; CIPARS has not, to date, included fish and shellfish. Since 2008, the CIPARS retail sampling infrastructure has been used to collect fresh and frozen raw salmon and shrimp. Enteric bacteria, including *Salmonella* and *E. coli*, have been recovered using standard protocols. *Salmonella* are serotyped; and minimum inhibitory concentrations are determined by the broth microdilution method using the Sensititre system and the NARMS susceptibility plate, CMV1AGNF. Isolates resistant to ampicillin have been screened for major β -lactamase genes by PCR. For samples collected between April 2008 and August 2010, *Salmonella* (serovars Weltevreden, Saintpaul, Virchow, Wandsworth, Paratyphi B. var Java, Paratyphi B., Brunei, Newport, and Bootle) were recovered from 4% of shrimp samples. Resistance in *Salmonella* was rare. Recovery of *E. coli* was 35% in shrimp and 19% in salmon. In generic *E. coli*, resistance to ciprofloxacin was found in 3% of shrimp isolates, and nalidixic acid resistance in isolates from both shrimp (6%) and salmon (2%). Amoxicillin-clavulanic acid resistance was found in 6% of salmon and 1% of shrimp isolates (always in combination with ampicillin and one or more cephalosporins). β -lactamase determinants were found in *E. coli* from shrimp and salmon including *bla_{CMV}*, *bla_{CTX-M}*, *bla_{PSE-1}* and *bla_{TEM}*, and most occurred in combination with genes conferring resistance to other drug families. The presence of ESBLs and cephamycinases emphasizes the importance of prudent use of antimicrobials in aquaculture in the context of anthropogenic dissemination of antimicrobial resistance and public health.

FOOD AND ENVIRONMENTAL SAFETY POSTERS

049P

Presence of *Listeria Monocytogenes* and *Salmonella* in ready to eat meats from processing plants and retail outlets in North Dakota.

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The US Department of Agriculture/ Food Safety and Inspection Service (USDA/ FSIS) has been conducting a regulatory microbiological testing program on ready to eat (RTE) meats since 1983. While the trend for the last three years indicate a decrease in the percent positives for *Salmonella* the *Listeria*, prevalence has remained almost constant. This study investigated presence of *Listeria monocytogenes* and *Salmonella* in RTE meats from processing plants and retail outlets in North Dakota. Since March 2010, samples of assorted RTE meat products (hot dogs, sausages, sliced meats) were obtained from processing plants in ND under the state meat inspection program; others were purchased from retail outlets in ND. The USDA/FSIS protocols for isolation of *Salmonella* and *Listeria monocytogenes* in RTE meats was followed. Of 59 meat samples processed so far 10/59 (16.9%) tested positive for either *Listeria* or *Salmonella*; 5/59 (8.5%) and 5/59 (8.5%) tested positive for *Listeria* and *Salmonella*, respectively. Of the 10 meats that tested positive for either *Listeria* or *Salmonella* 4 and 6 meats were from retail stores and processing plants, respectively. Among *Listeria* positive samples, 1/31 (3.2%) were from sliced deli meats, 3/18 (16.7%), hotdogs, and 1/10 (10%) sausages. Among *Salmonella*, positive samples, 1/18 (5.6%) were from hotdogs and 4/10 (40%) from sausages; all 31 sliced deli meats tested negative for *Salmonella*. Further analysis is underway to determine if presence of *Salmonella* and/or *Listeria* in RTE meats is significantly influenced by source and type of RTE meat. These data indicate presence of both *Salmonella* and *Listeria* in RTE meats in ND underscoring the need for improved monitoring of food safety measures in both processing plants and retail stores.

050P

Characterization and distribution of *Salmonella* from egg layer and pullet grower operations in Ontario, Canada, by MLVA molecular typing.

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Purpose: In Canada, the egg industry performs mandatory testing to determine the presence of *Salmonella* in the production environment, with efforts centered on the identification of *S. Enteritidis*. The control of *Salmonella* at a farm level requires tools allowing for the precise identification of all strains at the subspecies level. In partnership with the Poultry Industry Council, we have established a project to characterize environmental *Salmonella* isolates obtained from egg layer and pullet grower operations across Ontario by Multiple Loci Variable Number Tandem Repeat Analysis (MLVA). We are using MLVA data to examine the persistence of strains in the production environment as well as investigate the movement of *Salmonella* by linking MLVA data with GIS information and flock movement data.

Methods: A total of 10,000 samples are being collected from approximately 480 egg producers and pullet growers. During the lifetime of each flock, the environment is sampled at 10 and 60 weeks of age at specific sampling sites and tested for *Salmonella* using an automated immunomagnetic separation culture method. MLVA analysis is then performed in multiplex PCR assays using fluorescent-labeled forward primers based on selected specific loci. The PCR fragments are then separated using an ABI 3730 Genetic Analyzer and the fragment data is analyzed using GeneMapper and BioNumerics software programs to determine the relationship among different isolates. In addition, isolates displaying unique MLVA genotypes are undergoing serotyping and phage typing at the laboratory for Foodborne Zoonoses, Public Health Agency of Canada.

Results: From 7,500 samples tested, 1,234 *Salmonella* isolates have been isolated for MLVA analysis. The distribution of MLVA *Salmonella* genotypes will be presented.

Conclusions: This research will generate ongoing epidemiological data for the Ontario egg industry and will build a *Salmonella* isolates database linking molecular typing to classical serotyping and phage typing data to more effectively monitor and control *Salmonella* in the production environment.

051P

Comparison of RT PCR with three culture methods for detection of *Salmonella* from poultry environmental samples.

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Purpose: This study was conducted to compare RT PCR versus several different culture methods for detection of *Salmonella* in samples collected from poultry environments. Methods: One hundred and ten environmental samples were collected from poultry facilities using drag swabs or hydrosponges and placed in whirlpak bags. Tetrathionate (Tet) containing 2 ml iodine per 100 ml Tet was added to all samples and incubated overnight at 42°C. RT-PCR was conducted using aliquots of Tet from these samples after the primary enrichment (PE). Aliquots of PE were also streaked onto selective media which included brilliant green w/novobiocin (BGN) and XLT4. 100 µl aliquot of Tet from each sample was also inoculated into semisolid MRSV media plates and incubated at 42°C for 20-24 hours followed by inoculating into Gram Negative (GN) broth and incubating at 35°C for 6-8 hours. After initial plating of primary enrichment specimen bags containing Tet, bags were then incubated at ambient temperature for 5-7 days followed by a 1 ml aliquot of each sample being transferred to a secondary enrichment media (Delayed secondary enrichment or DSE) Tet broth and incubated for 16 - 22 hours at 42°C. After incubation samples were then streaked onto BGN and XLT4. Suspect colonies were selected and tested with several biochemical reactions. Positive *Salmonella* isolates were confirmed by *Salmonella* serogrouping. Results: *Salmonella* was isolated from PE in 45 samples, from DSE in 49 samples, from MRSV in 52 samples and from any culture method in 68 samples. *Salmonella* was detected by RT PCR in 83 samples. There was agreement between PE and RT PCR in 68 samples, between DSE and RT PCR in 74 samples, between MRSV and RT PCR 79 samples, and any culture method and RT PCR in 91 samples. There were 17 samples positive by RT PCR but negative by any culture method. Only two samples had *Salmonella* detected by at least one culture method but were negative by RT PCR. Conclusions: In this study RT PCR appears to be a reliable test method to detect *Salmonella* from poultry environmental samples following enrichment and may be a useful tool to screen large numbers of samples. Thus reducing time, labor and expense associated with traditional culture methods.

GASTROENTERIC DISEASES POSTERS

053P

Protective effects of a transgenic carrot vaccine on piglet diarrhea.

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Purpose: Diarrhea caused by enterotoxigenic *Escherichia coli* (ETEC) K88ac is a common problem among neonatal and newly weaned piglets. The purpose of this study was to evaluate whether a transgenic carrot vaccine could induce the K88-specific immune response and have the protective effect against enterotoxigenic *Escherichia coli* (ETEC) K88ac in piglets through the maternal antibody.

Methods: The K88ac antigen gene (pilin gene) was isolated from the K88ac genomic DNA using PCR. The pilin gene constructed into pGA748 was introduced via *Agrobacterium tumefaciens* to the explants of carrot hypocotyls to get the transgenic cell line. PCR and western blotting was performed to confirm the homogeneity between K88ac antigen and transgenic cell line. The intensity of each protein were quantified using Melanie II program (GeneBio). Sows that have been selected randomly from a farm in Korea were assigned to 3 groups: control (5 sows) and group A (5 sows) was inoculated with a non-transgenic carrot and transgenic carrot vaccine at 2 weeks and 4 weeks ante partum orally, respectively, and group B (5 sows) was vaccinated with commercial vaccine according to the manufacturer's instructions. After 7-days of lactation, every 5 piglets selected randomly from sows of each group were challenged with 1×10^8 CFU/ml ETEC K88ac.

Results: ETEC K88ac antigen gene introduced in the cell lines was confirmed by PCR (870bp) and western blotting with pilin (28kDa) antiserum. In the quantitative analysis with Melanie II, the amount of pilin protein was from 91.1 ul/g to 106.1 ul/g. Group B had the lowest mean fecal consistency score on day 1 and 7. Group A and B compared with control tended to have increased villi height, reduced crypt depth, broadened villi width and increased villus: crypt ratio in the intestinal morphology. In particular, the concentration of IgA and IgG against ETEC K88ac was higher in group A and B in serum and colostrums, compared to control.

Conclusions: Based on the results, it was concluded that the transgenic carrot vaccine in sow per oral may have an effect on preventing piglet diarrhea as good as commercial recombinant vaccine.

054P

Application of a 'FaeG-FedF-LT_B' adhesin-toxin fusion antigen in vaccine development against enterotoxigenic *Escherichia coli* associated diarrhea in pigs.

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Enterotoxigenic *Escherichia coli* (ETEC) strains expressing K88 (F4) or F18 fimbriae and enterotoxins are the main source associated with diarrhea in weaned pigs. Post-weaning diarrhea (PWD) causes substantial economic loss to the swine producers, yet no effective vaccines have been developed to control or prevent PWD. It is believed that only vaccines inducing anti-toxin and anti-adhesin immunity could provide broad protection against ETEC associated diarrhea. In this study, we genetically fused a part of the *faeG* gene coding the FaeG major subunit of K88ac fimbriae, part of the *fedF* gene coding the FedF subunit of F18 fimbriae, and the *eltB* gene coding B subunit of the heat-labile toxin (LT) for adhesin-toxin chimeric gene. This adhesin-toxin chimeric gene was cloned into a pBAD vector and expressed in TOP10 *E. coli* cells. The expressed fusion protein was purified and used to immunization studies to assess anti-K88, anti-F18 and anti-CT antigenicity. Vaccine candidacy of this adhesin-toxin fusion in against porcine ETEC diarrhea was preliminarily evaluated in an animal challenge study.

055P

Genotypic comparison of virulent extraintestinal pathogenic *Escherichia coli* isolates causing fetal pneumonia in animals with those from healthy human.

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Extraintestinal pathogenic *E. coli* strains (ExPEC) have been implicated in a variety of diseases in humans such as urinary tract infection (UTI), pneumonia, neonatal meningitis and septicemia. In animals, this pathotype has been associated with diseases including colibacillosis in poultry, bovine septicemia, UTI and hemorrhagic pneumonia. The objective was to compare the genotypic and phylogenetic profiles of *E. coli* that caused fetal pneumonia in dogs, cats, horses and a tiger with *E. coli* isolated from a healthy human and a healthy skunk possessing similar virulence profiles as those of pneumonia-causing isolates. The isolates were selected from the collection of *E. coli* Reference Center. The serotyping and virulence profiling of 25 genes were performed using standard techniques. The phylogenetic grouping, multilocus sequence typing (MLST) and pulsed-field gel electrophoresis (PFGE) were performed as reported earlier. *E. coli* from the animals with pneumonia belonged to O6:H31 or O4:H5 and carried 14-15 virulence genes that are known to be associated with virulent ExPEC strains. The ExPEC isolate from the healthy human belonged to O4:H5 whereas the skunk isolate was O6:H31, and both carried 13 of the same virulence genes as those *E. coli* from the sick animals. The common virulence genes carried by all isolates were *cnf1*, *papGIII*, *papA*, *papC*, *fimH*, *hlyD*, *iroN*, *fyuA*, *ompT*, *uidA*, *usp* and PAI. All the isolates belonged to the phylogenetic group B2. PFGE analysis showed that these isolates were not related to each other. Since virulence gene profiles of *E. coli* from sick animals were identical to those from healthy human and skunk, it is likely that the pathogenicity of ExPEC strains is not merely determined by the possession of a set of virulence genes. The severity of the disease may be linked to the expression of virulence genes *in vivo* and/or the level of host immunity. Level of expression of virulence genes *in vitro* may shed light on the pathogenic potentials of the ExPEC isolates.

056P

Morphological and Structural Changes in Colon of adult FVB Mice Infected with *Citrobacter rodentium*.

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Citrobacter rodentium causes transmissible murine colonic hyperplasia and is used as a model for enteropathogenic and enterohemorrhagic *Escherichia coli* infections. While *C. rodentium* causes little or no mortality in most mice strains, the FVB and C3H strains develop fatal colitis. The objective of this study was to evaluate morphological and physiological changes in colonic epithelium of adult FVB mice (12 weeks of age) following intracecal inoculation with *C. rodentium*. Colons of infected mice were colonized heavily by bacteria, which also caused effacement lesions. Small numbers of bacteria were also translocated into the epithelial cells, mesenteric lymph nodes, spleen and liver. Tight junctions were disrupted in the colonic epithelium of infected mice as indicated by a punctate pattern of occludin labeling. Occludin and actin pedestals were colocalized subjacent to the attached bacteria. Pedestal formation and intercellular and intracellular invasion of bacteria were also observed with electron microscopy. Despite these structural changes, there were no significant changes in transepithelial electrical resistance and mice did not develop clinical signs of the disease.

GASTROENTERIC DISEASES POSTERS

057P

Identification of *Helicobacter suis* in pig-producing regions of North America.

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Purpose: *Helicobacter suis* is a recently isolated and characterized bacterium (Baele, *et al.*, *Int J Syst Evol Microbiol.* 2008. 58:1350), associated with gastric ulcers in swine. To date, *H. suis* has only been isolated in Europe. Since this organism is very difficult to isolate by culture, little is known about its distribution in U.S. pigs.

Methods: In order to estimate the relative prevalence of *H. suis* in the U.S., we developed genus and species specific quantitative-PCR methods to quantitate *H. suis* in porcine stomachs. These primer-probe sets were tested with samples (gastric mucosal scrapings) from pigs in three geographic regions of the U.S., including midwest (Iowa), south (Oklahoma) and east (North Carolina).

Results: Of a total of 70 samples, between 35% and 80% of pigs, depending on the region were positive for *H. suis* DNA.

Conclusions: These results suggest that *H. suis* is common and widespread in the U.S. pig population. This assay can be used to determine the prevalence of *H. suis*, its impact on pig health and production and whether it may be an important target for therapeutic and prophylactic interventions.

058P

Experimental infection of swine with *Helicobacter suis*.

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Purpose: *Helicobacter suis* is a recently isolated and characterized bacterium (Baele, *et al.*, *Int J Syst Evol Microbiol.* 2008. 58:1350) found in swine throughout the world. It has been associated with gastric ulcers and with decreased weight gain. *H. suis* is difficult to isolate and grow, making experimental models of infection challenging.

Methods: In this study, medicated early weaned (MEW) pigs were challenged one or two times with 10^9 *in vitro*-grown *H. suis* (European isolate HS5; Prof. R. Ducatelle, Ghent University) at 4 and 5 weeks of age and were fed normal or finely ground feed with or without added fructose and with or without intermittent fasting following challenge. Clinical parameters (including body weight) and immunological response (serology) were monitored for 8 weeks following challenge. At necropsy, stomachs were scored for gastric lesions and *H. suis* colonization was determined by quantitative PCR.

Results: All pigs receiving challenge organisms were positive for *H. suis* colonization at necropsy. In addition, many pigs that were not challenged with *H. suis* became positive during the course of the study, suggesting possible cross-contamination between pigs during the study (and the contagious nature of this organism). There was a serological response to challenge in some pigs. There was no obvious correlation of gastric lesions with *H. suis* challenge since many non-challenged pigs also developed gastric lesions. Although this study was not designed to statistically analyze the effect of *H. suis* challenge on body weight, there was a numerical decrease in mean weight gain in challenged pigs.

Conclusions: These results confirm pigs can be experimentally infected with *H. suis*, and suggest a complex relationship between dietary stress and *H. suis* colonization and both gastric lesions and weight gain.

059P

Deletion of glucose-inhibited division gene (*gidA*) alters the morphological and replication characteristics of *Salmonella enterica* serovar Typhimurium.

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Salmonella is an important food-borne pathogen that continues to plague the United States food industry due to consumption of contaminated food. Identification and characterization of bacterial factors involved in *Salmonella* virulence and its regulation would help develop effective strategies for controlling salmonellosis. Studies have indicated that deletion of glucose-inhibited division gene (*gidA*) significantly altered biological and pathogenic abilities of several bacterial pathogens such as *Aeromonas hydrophila*, *Escherichia coli* and *Pseudomonas syringae*. We have shown deletion of *gidA* significantly alters *S. Typhimurium* virulence in both *in vitro* and *in vivo* models of infection, and mice immunized with the *gidA* mutant strain were protected from a lethal dose challenge of wild-type (WT) *S. Typhimurium*. Furthermore, we have shown that deletion of *gidA* displays a filamentous morphology, when compared to the bacillus nature of WT *S. Typhimurium* cells. In this study, scanning electron microscopy was used to further characterize the filamentous morphology of the *gidA* mutant, and our results confirm the filamentous nature of the *gidA* cells. DAPI-stained samples of the *gidA* mutant and WT *S. Typhimurium* strains were analyzed by fluorescence microscopy. Our results indicated a defect in chromosome segregation in the *gidA* mutant, and these findings were confirmed by transmission electron microscopy. We used a microarray to determine the differential gene expression in the *gidA* mutant when compared to the WT *S. Typhimurium* strain. Our transcriptome analysis revealed several genes involved in cell division were altered in the *gidA* mutant, and alteration in these genes was confirmed by real-time RT-PCR. Specific antibodies were used to characterize proteins encoded by these genes. Our results indicated a significant alteration in the expression and localization of these proteins in the *gidA* mutant when compared to the WT *S. Typhimurium* strain. Taken together, our data indicates GidA plays a significant role in the cell division of *S. Typhimurium*.

GASTROENTERIC DISEASES POSTERS

060P

Comparison of selenite and Rappaport-Vassiliadis enrichment methods for isolation of *Salmonella* from dairy cattle naturally infected with *Salmonella* Newport MDR-AmpC.

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Purpose: To compare overnight selenite (SEL) enrichment to a modified ISO6579:2002 culture method using Rappaport-Vassiliadis (RV) broth for isolation of *Salmonella* and describe fecal shedding in dairy cattle following a natural outbreak of salmonellosis caused S. Newport MDR-AmpC.

Methods: A cohort of 55 dairy cows were followed longitudinally. Individual fecal samples were obtained once every 2 weeks for 10 weeks then once monthly for 4 months. Each sample was divided and cultured using SEL and RV methods. Isolates presumptively identified as *Salmonella* were serogrouped.

Results: There were 461 samples, 219 (47.5%) were positive for *Salmonella*; 156 (71.2%) with both SEL and RV, 60 (27.4%) with RV but not SEL, only 3 (1.4%) with SEL but not RV. All positive samples had at least 1 isolate serogrouped; of 375 isolates serogrouped, 350 (93.3%) were C2 and 25 (6.7%) poly-O. Duration of fecal *Salmonella* shedding ranged from 0 to 24 weeks. Average duration of shedding was 6.25 (SD 5.01, SE 0.68) and 9.02 (SD 6.29, SE 0.85) weeks for SEL and RV, respectively; a significant difference by both parametric (paired t-test; $P < 0.001$, $\beta = 0.20$) and non-parametric (Wilcoxon signed-rank test; $P < 0.001$) methods. There was no significant difference in average duration of shedding across lactation groups 1-7 (Kruskal-Wallis test; SEL $P = 0.15$, RV $P = 0.21$); nor was there a difference between heifers and cows (Mann-Whitney U test; SEL $P = 0.91$, RV $P = 0.30$). Survival curves were generated using Kaplan-Meier analysis. During the study period, 47/55 (85.5%) and 43/55 (78.0%) cows achieved a culture negative status event for SEL and RV culture methods, respectively. The majority 32/43 (74%) and 39/47 (83%) of events occurred within the first 10 weeks.

Conclusions: When used in a modified ISO procedure, RV was more sensitive than SEL in isolation of *Salmonella* from dairy cow feces. SEL and RV used in parallel would not increase sensitivity beyond that of RV alone. While RV was more sensitive, the cost of the procedure may outweigh this benefit. As documented for other *Salmonella* serovars, the majority of infected cattle were culture negative in less than 4 months following the outbreak; however, 1 cow remained positive for 40 weeks.

061P

Phylogenetic analysis of the 3D region of porcine kobuviruses detected from Korean diarrheic pigs.

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Purpose: Picornaviruses are positive single-stranded RNA viruses that cause a variety of important disease states in humans and animals. Kobuvirus is a newly recognized genus in the family *Picornaviridae*. Among the kobuviruses, porcine kobuvirus has been found recently, and consequently information about the virus is lacking. In the present study, to a better understanding of genetic background of porcine kobuvirus, we conducted sequence and phylogenetic analysis.

Methods: Generic kobuvirus primers (UNIV-kobu-F/UNIV-kobu-R), targeting the 3D region (corresponding to nt 7334-7550 of the porcine kobuvirus S-1-HUN; 217 bp) of all kobuvirus species (Aichi virus, bovine kobuvirus, and porcine kobuvirus), were used for the detection of porcine kobuvirus from Korean diarrheic pigs, and PCR was performed using a protocol described previously. Seventeen representative strains amplified strongly by RT-PCR were selected, sequenced, and analyzed for their evolutionary relationships with other kobuvirus reference strains.

Results: Sequence analysis of the 3D region of porcine kobuviruses showed that all of selected strains have high sequence identities with the 3D region of the porcine kobuvirus reference strains reported previously in Hungary (90.1-95.5%), China (90.7-97.1%), Thailand (87.0-98.0%), and Japan (88.3-96.7%). On the other hand, they have relatively low sequence identities with Aichi virus (63.0-66.4%) and bovine kobuvirus (67.0-70.5%). Phylogenetic analysis of 3D nucleotide sequences of our kobuvirus strains together with those of published porcine kobuvirus reference strains as well as Aichi virus and bovine kobuvirus showed that all of 17 strains belonged to porcine kobuvirus and formed a tight cluster in a monophyletic branch with other previously reported porcine kobuvirus strains. In addition, the sequences of kobuvirus obtained from pigs with the age of 6 weeks or ≤ 22 weeks old were not different from those of ≤ 3 weeks old.

Conclusions: The present study helped elucidate phylogenetic relationships of Korean porcine kobuviruses with other kobuvirus reference strains.

062P

Identification of the receptor-binding domain (RBD) of the porcine epidemic diarrhea virus spike protein

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Porcine epidemic diarrhea virus (PEDV) infection causes acute enteritis with lethal watery diarrhea leading to death with a high mortality in piglet. As with the other members in group 1 coronaviruses, PEDV also utilizes the host aminopeptidase N (APN) as the major cellular receptor for entry to target cells. The coronavirus spike (S) protein is known to interact with the cellular surface for viral attachment and the S1 domain of all characterized coronaviruses contains a receptor-binding domain (RBD) that mediates a specific high-affinity interaction with their respective cellular receptors.

Although the RBDs of several coronaviruses have been mapped, the location of the PEDV RBD remains to be defined. As a first step toward identifying the region in the S protein of PEDV that is critical for recognition with the cellular receptor, we generated a series of S1-truncated variants and examined their abilities to bind to the porcine APN (pAPN) receptor. Our data indicated that the N terminus of the S1 domain is required for pAPN association. The results from the present study may help our understanding of the molecular interactions between the PEDV S protein and the pAPN receptor.

IMMUNOLOGY POSTERS

063P

The US Veterinary Immune Reagent Network: Update on reagents for the horse.

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The US Veterinary Immune Reagent Network (US-VIRN, www.vetimm.org)

aims to develop new tools for ruminants, swine, horse, poultry and aquaculture species to improve immunological research in infectious diseases and animal health, and to contribute to new vaccine development strategies and food safety. For the horse, several new reagents were developed including recombinant cytokines and chemokines, and monoclonal antibodies (mAbs) to cytokines and cell surface molecules. Recombinant cytokines and chemokines were produced by Kingfisher Biotech in a yeast system. Equine recombinant IL-1 β , IL-2, IL-4, IL-6, IL-13, IL-17A, GM-CSF, CCL2, CCL3, CCL5, CCL11, CXCL9 and CXCL10 were produced and can be obtained from Kingfisher (www.kingfisherbiotech.com). Monoclonal antibodies (mAbs) to cytokines and cell surface markers were produced at Cornell University. For cell surface molecules, recombinant proteins were expressed in mammalian IgG or IL-4 fusion protein system and used for immunization of mice. Fully characterized mAbs with proven specificity to the native protein were developed to equine IL-4, IL-10, IFN- α , IL-2, CCL2, CCL3 and to equine CD14 and CD23. These mAbs can be obtained from Cornell University

(<http://www.cctec.cornell.edu/> or bw73@cornell.edu). Additional mAbs that are either in the production or characterization processes include reagents to IL-1 β , IL-5, IL-6, IL-17A, IFN- β , GM-CSF, CCL5, CCL11, and CD25, CD28, CD40, Fc ϵ R1 α , TCR α , TCR δ , and TCR γ .

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064P

Swine toolkit progress for the US Veterinary Immune Reagent Network.

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The US Veterinary Immune Reagent Network (vetimm.org) was established to address the lack of sufficient immunological reagents specific for ruminants, swine, poultry, equine and aquaculture species. For pigs major progress has been made in the last year. Recombinant chemokines CCL2, CCL3L1, CCL4 CCL5 and CCL20 were expressed in *Pichia* and shown to be bioactive using chemotaxis or upregulation of marker expression. Monoclonal antibodies (mAb) to CCL2 have been produced and a sensitive fluorescent microsphere, Luminex bead, immunoassay developed. Immunizations for others are in progress. The cytokines, interleukin-6 (IL-6), IL-15, IL-17A, IL-17F, interferon-alpha (IFN α) and IFN γ have been expressed; bioactivity of most have been affirmed, testing of the others is underway. Hybridoma fusions for mAb to CCL3L1 and IL-17A are underway. At Cornell Univ. a fusion protein expression system for swine T cell receptors, TCR $\alpha\beta$, was used to generate material for immunizations and hybridoma fusions expected in fall 2010. Additional fusions will target IFNAR and NK cell markers. A priority reagent development list and progress update for swine is available at vetimm.org as are all bioassay methods and gene sequences. Since many swine cytokine and CD reagents are available commercially the Network website includes a listing of those reagents and is regularly updated. Our goal is to produce reagents that function in ELISA, Luminex assays, ELISPOT and flow cytometric applications. Products developed in this proposal are available to collaborators and have been made commercially available using non-exclusive licenses, including Kingfisher Biotech, Inc. <http://www.kingfisherbiotech.com/>. This project was funded by USDA NIFA proposal #2006-35204-16880, renewal #2010-65121-20649, US DHS IAA #HSHQDC10X00021 and USDA ARS funds.

065P

Comparison of agreement rate between developed C-ELISA and commercial C-ELISA assay for serological diagnosis of bovine Brucellosis.

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Serodiagnostic tests for brucellosis are mainly based on detection of antibodies developed against lipopolysaccharide (LPS) of cell. Although LPS has a limitation to discriminate false positive reaction accurately, but LPS is one of the most valuable antigens to diagnose bovine brucellosis. In the present study, we developed a competitive ELISA (*Brucella*-Ab C-ELISA) using two monoclonal antibodies (8C10 and 8G12) that were capable of discriminating *B. abortus* and *Y. enterocolitica* infection. To establish the condition of *Brucella*-Ab C-ELISA assay, ELISA plates were coated with three different antigens such as LPS of *B. abortus*, 8C10+LPS, and 8G12+LPS. In addition, we compared the reactivity with 8C10-HRP and 8G12-HRP conjugates using the reference sera. After selection of coating antigen and conjugate, *Brucella*-Ab C-ELISA assay was estimated by comparing with commercial competitive ELISA assay (SVANOVIR *Brucella* Ab C-ELISA) and TAT (tube agglutination test) using positive (467) and negative sera (635) that were verified by Rose-Bengal test. For set-up of C-ELISA conditions, we selected LPS antigen and 8C10-HRP as a plate coating antigen and a conjugate, respectively. When compared with commercial competitive ELISA assay, the agreement rate between developed *Brucella*-Ab C-ELISA and commercial C-ELISA was 98.54% (1,086/1,102). In addition, the agreement rate between *Brucella*-Ab C-ELISA or SVANOVIR *Brucella* Ab C-ELISA and TAT were all 94.01% (1,036/1,102). These results suggest that the newly developed *Brucella*-Ab C-ELISA might be used as an effective alternative diagnostic tool as a single or combination assay with TAT for serodiagnosis of bovine brucellosis.

066P

Development of specific antigens for serodiagnosis of *Mycobacterium bovis* using latex bead agglutination assay.

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Introduction: *Mycobacterium bovis* is a causative pathogen of bovine tuberculosis that is known to be one of the important zoonoses. Generally bovine tuberculosis has been diagnosed at antemortem using various methods such as intradermal skin test (IST), interferon-gamma assay (IFN- γ), and ELISA. However, the diagnosis of bTB results in tremendous economic loss and is considered to be a troublesome work because it needs long time to confirm bovine tuberculosis and the acquirement of fresh blood and tissues of the animal. To develop a rapid and efficient diagnosis method of bovine tuberculosis, the present study was to search for specific antigens from the proteins of *M. bovis* for their use in latex bead assay (LBAA).

Materials and Methods: The 9 peptides such as ESAT-6 (P2, P3), MPB83 (P4), MPB70 (P5, P9), MPT64 (P6, P7, P8) and GlcB(P10) were isolated from the proteins obtained after harvesting of *M. bovis*. Their efficiency as a specific antigen for application of LBAA were determined through agglutination, specificity, and sensitivity test. Each selected peptide conjugated with carboxylated polystyrene nanospheres from Bangs Labs Inc was used as the

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antigens in LBAA. Also, *M. bovis* infected serum was used as a positive control, while the commercial fetal bovine sera and non-infected cattle sera were employed as negative sera.

Results: Among 9 peptide candidates, peptides (P5, P7) showed significant difference in the capability of agglutination when compared with positive sera and negative sera. In the check broad test performed with MPT64 (P7), the optimal amount of the antigen and the starting time of agglutination appeared to be 20 µl and 1 min, respectively. Moreover, the MPT64 (P7) was found to have the shortest starting time of agglutination among 9 peptide candidates and the LBAA using MPT64 (P7) showed 100% of sensitivity and 72.7% of specificity when they were estimated with positive(n=20) and negative(n=11) sera.

Conclusions: These results demonstrate that P7 has the potential of a specific antigen for LBAA and the its application in LBAA seems to allow a rapid diagnosis of bovine tuberculosis even by farmers or local veterinarians.

067P

Development of a skin test to map equine infectious anemia virus (EIAV) envelope-specific immune responses *in vivo*.

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Equine infectious anemia virus (EIAV) is an equine lentivirus which shares structural similarity with HIV-1. EIAV infection is characterized by three infection phases: acute, chronic and inapparent carrier states. In the first year of infection, most infected horses show recurring disease cycles. After that, the infected horses enter the inapparent carrier state in which virus replication is tightly controlled by the immune system as evidence by the fact that immunosuppressive drugs can induce the recurrence of disease. This naturally gained protective immunity makes EIAV a model for lentivirus vaccine development. Characterization of cellular immunity is usually done by *in vitro* assays using peripheral blood mononuclear cells (PBMC). Considering that less than 2% of lymphocytes reside in blood, PBMC responses may not fully reflect overall cellular immunity. Therefore, we developed a skin test to monitor the EIAV envelope specific cellular immunity *in vivo*.

Two infected and two naïve horses were included in this study. Forty-four 20-mer peptides, representing the entire surface unit protein of EIAV, were used to construct peptide pools. All horses were intradermally injected with 10µg peptide pools in 0.1ml saline and 0.1ml saline was injected alone as a negative control. After 48h, palpable infiltrations were measured before obtaining 2mm and 4mm punch biopsies from the site. The 2mm biopsies were homogenized, and total RNA was isolated for determination of gene expression by real-time PCR. The 4mm skin biopsies were formalin-fixed, paraffin-embedded, and later stained with a monoclonal antibody to CD3.

There were no palpable responses to the peptide pool injection sites in two naïve horses. By contrast, palpable responses were detected in the two infected horses. IHC staining results showed that a large portion of cells recruited to the injection sites were CD3 positive cells. Additionally, the biopsy samples had increased gene expressions of granzyme B and perforin, which are two markers of cytotoxic T cells. Interferon-γ was also detected in the biopsy samples.

These results indicate that skin testing can be used to demonstrate EIAV peptide-specific immune responses *in vivo*.

068P

Do the criteria used to interpret the microscopic agglutination test (MAT) for the diagnosis of canine Leptospirosis need to be changed?

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Purpose: The most common test used for the diagnosis of canine leptospirosis is the MAT which detects antibody to leptospira. MAT is relatively sensitive and specific and often includes serovars *L. autumnalis*, *bratislava*, *canicola*, *grippityphosa*, *hardjo*, *icterohaemorrhagiae*, and/or *pomona*. Recently, two 4-way canine leptospira bacterins have been licensed to replace the 2 way bacterins in use since the 1970's. The 2 way vaccines contained the two most important canine serovars, *L. canicola*, in which the dog serves as the natural reservoir, and *L. icterohaemorrhagiae*. The two serovars added were *L. grippityphosa* and *L. pomona*, both canine pathogens. More dogs are being vaccinated with 4 way vaccines than with 2 way products. Due to the additional serovars, the new vaccines cause more cross reactivity and create higher titers on MAT to serovars like *L. autumnalis* and *L. bratislava* that are not in the vaccines.

Method: we used the MAT to determine if:

1. It would reliably detect the infecting serovar
2. The 2 and 4 way vaccines induced antibody to all of the serovars tested
3. A titer of ≥ 800 on a single sample could be considered diagnostic of infection
4. Experimental monovalent vaccines induced titers which cross reacted with multiple serovars and/or induced titers that would be incorrectly interpreted as infection rather than vaccination
5. Titers to *L. autumnalis* and/or *L. bratislava* affect accuracy and value of the MAT

Results: New criteria we propose for diagnosis of canine leptospiral disease are:

1. MAT titers to only serovars *L. canicola*, *L. grippityphosa*, *L. icterohaemorrhagiae*, and/or *L. pomona* should be used for the diagnosis of canine leptospirosis
2. With a single sample, a titer should be ≥ 1600 for one or more of these 4 serovars
3. When acute and convalescent samples are available, the increase in titer to one or more of the 4 serovars should be an 8 fold or greater increase
4. An acute titer of ≥ 1600 would constitute a positive sample, even if the convalescent sample did not show an 8 fold increase

Conclusions: We believe these changes will significantly increase the value of the MAT as a method to diagnose canine leptospirosis.

069P

Mycobacterial immunodominant antigens ESAT6 and CFP10 improve tuberculin skin test specificity in cattle naturally infected.

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The cornerstone of tuberculosis (TB) control in cattle and other species is the accurate detection and removal of animals infected with *Mycobacterium bovis*; however, the limitations in the specificity of tuberculin testing result in a failure to differentiate between *M. bovis* infected animals and animals exposed to non-pathogenic environmental mycobacterial species. The ESAT-6 and CFP-10 antigens, are strongly recognized in TB infected cattle, and they do not elicit a response in cattle without infection. Besides, they are absent in most of environmental mycobacterial species, so its use can be an

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alternative to PPD tuberculin in the development of more specific skin diagnostic test in cattle and other species.

The aim of the current study was to assess the potential of an ESAT-6 and CFP-10 protein cocktail in a skin-test format in naturally infected cattle, with different tuberculosis prevalences.

A pilot study was designed including 10 *M. bovis* infected cattle from a herd with a prevalence higher than 30% and 10 non exposed cattle from a TB-free herd. An IFN- γ whole blood test and a PCR from nasal swabs were performed in order to confirm the presence of *M. bovis*. The protein cocktail was tested at different dose concentration of 5, 10, 15 μ g for each protein. Skin induration at inoculation sites was measured using callipers, size was recorded prior to, and every 24 hours for 4 days.

Skin responses to the protein cocktail peaked after 72 hours. *M. bovis* infected animals showed high positive skin responses to PPD-bovine, but skin responses to protein cocktail different dose concentrations were significantly lower. The best skin response to the protein cocktail was obtained with 10 μ g, the mean was 6.3 mm, compared to almost 12 mm seen in reactions to PPD-bovine. The non exposed animals did not respond to either cocktail or PPD-bovine; however, they responded towards PPD-avian (2.4mm).

After assessment of protein cocktail dose concentration and skin measurement time, the protein cocktail was tested in 4 herds with different tuberculosis prevalences. Our data shown that the ESAT-6 and CFP-10 protein cocktail improve the specificity of the skin test without affecting the sensitivity.

070P

The probiotic strain *Lactobacillus rhamnosus* GG has anti-viral effects against three canine viruses

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Purpose: Probiotics such as *Lactobacillus rhamnosus* GG are known to stimulate the immune system and act against pathogenic organisms. The live probiotic bacteria, their secreted products or their cellular components indirectly or directly stimulate the immune system and/or affect the growth of pathogens. In this study, we examined whether the cell lysate or the culture supernatant of the probiotic strain *L. rhamnosus* GG has an antiviral effect against canine adenovirus (CAV-1), distemper virus (CDV) and parvovirus Type 2 (CPV-2) in cell cultures.

Methods: *L. rhamnosus* GG was grown overnight to 10^9 /ml and cell-free culture supernatant was prepared. The pellets were lysed by sonication after repeated freeze-thawing. Cell monolayers of MDCK (for studies on CAV-1 and CPV-2) and vero (for studies on CDV) cells were pretreated with either the probiotic culture supernatant or its cell lysate and incubated for 2h. Control plates were incubated with the appropriate media. Cells were then infected with the respective viruses and further incubated for 2h, for RNA extraction, or 72h, for examining cytopathic effects and cell survival assay by MTT test. At this time point, viruses were also collected to determine the viral titer using the TCID₅₀ method. The titer of CPV-2 was determined by hemagglutination test and real time PCR. The studies were repeated three times.

Results: There was an increase in the cell survival and a decrease in the viral titer when monolayers were pretreated with the culture supernatant of the probiotic. Pretreatment of monolayers with the probiotic cell lysate did not result in protection of the cell lines from the viral infections. Further studies using real time PCR are in progress to determine the gene expression profiles of the probiotic-pretreated and untreated cells after viral infections. *In vivo* studies will follow.

Conclusion: Secreted metabolic products of *L. rhamnosus* GG have anti-viral effects.

071P

Serological survey of the southern Wisconsin raccoon population to *Leptospira*, canine parvovirus type 2, and canine distemper Virus.

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Wild and domestic animals are potential reservoirs for viral and bacterial pathogens. Raccoons and other wildlife species are reservoirs of *Leptospira* infection; with raccoons as the primary reservoir for *L. grippityphosa* and domestic dogs for *L. canicola*. *Leptospira* infections can cause various clinical symptoms (e.g. abortion, kidney disease, etc.). Domestic dogs are also reservoirs for viral pathogens (e.g. CPV-2, CDV, etc.) that infect other domestic and wild animals. Canine Parvovirus Type 2 (CPV-2), a stable DNA virus resistant to environmental change, induces infectious hemorrhagic enteritis. Canine Distemper Virus (CDV), an RNA virus that is less stable but able to remain infectious for long periods in the host, induces respiratory, gastrointestinal, and neurologic disease.

The present serological survey has examined exposure to bacterial (*Leptospira*) and viral (CPV-2, CDV) pathogens within the southern Wisconsin raccoon population. The presence of antibody to *Leptospira* was determined using microscopic agglutination tests (MAT). Antibody titers to CPV-2 and CDV were assessed with hemagglutination inhibition assays and viral neutralization assays, respectively. Positive *Leptospira* antibody titers (≥ 200) were as follows: 43% *L. grippityphosa*, 17% *L. canicola*, and 8% *L. pomona*. Further serology showed 61% of raccoons were positive (≥ 40) for CPV-2 and 46% were positive for CDV (≥ 4). Raccoons positive for *L. grippityphosa* antibody showed significantly higher numbers of CPV-2 positive antibody titers ($p < 0.05$). In addition, raccoons that were positive for CDV antibody also showed significantly higher numbers of CPV-2 positive antibody ($p < 0.001$). These results indicate raccoons primarily developed antibody to a *Leptospira* serovar of raccoon origin. CPV-2 antibody was a significant common co-positive, probably as a result of infection with raccoon Parvovirus, which is serologically identical to CPV-2. When compared to a seroprevalence study of Wisconsin raccoons conducted by our laboratory 25 years earlier, the percentages of animals positive for *Leptospira*, CDV, and CPV-2 was not different than those in the present study.

072P

Antibody and interferon- γ responses of pigs vaccinated with killed, adjuvanted H3N2 Swine Influenza Virus (SIV) vaccine.

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Purpose: Although the protective immune response to killed influenza vaccines is associated with development of a hemagglutination inhibition (HI) antibody response, cross-protection of swine influenza virus (SIV) vaccines has been demonstrated against strains in the absence of a cross-reactive HI response. Local mucosal antibodies and/or cell-mediated immune responses may also play a role in influenza protection.

Methods: We evaluated the development of H3N2-specific HI antibodies and interferon- γ (IFN- γ) secreting cells in pigs vaccinated intramuscularly (IM) or intradermally (ID) with killed H3N2 vaccines adjuvanted with Amphigen[®] or with proprietary aqueous adjuvants. Seventy two SIV-negative pigs were randomized to 9 treatment groups and vaccinated on Days 0 and 21. Pigs were bled for sera prior to each vaccination and on Days 28, 42, and 56.

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Heparinized blood samples, collected prior to and 7 days after each vaccination and on Day 56, were tested for H3N2-specific INF- γ secreting cells by ELISpot (Mabtech USA, Cincinnati, OH). This study (Study Report No. 4125W-06-09-303, Pfizer Inc) was conducted in accordance with Pfizer Animal Health's IACUC.

Results: Pigs vaccinated IM or ID with the Amphigen-adjuvanted vaccines developed significantly higher HI antibody titers than pigs vaccinated with the aqueous-adjuvant vaccines, or the placebo, at all post-revaccination time-points ($P \leq 0.01$). Groups vaccinated with the Amphigen-adjuvanted vaccines and some of the aqueous-adjuvanted vaccines had significantly higher numbers of SIV-specific INF- γ -secreting cells than the placebo vaccinates at the Day 28 and 56 post-revaccination time-points ($P \leq 0.05$).

Conclusions: These data indicate that a killed, adjuvanted vaccine can drive both the humoral and cell-mediated arms of the specific immune response to SIV.

073P

Immunologic responses to *Mycobacterium avium* subsp. *paratuberculosis* protein cocktail vaccines in a mouse model.

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Purpose: Johne's disease is a chronic granulomatous enteritis characterized by severe diarrhea, wasting and a decline in milk production caused by the bacterium *Mycobacterium avium* subsp. *paratuberculosis* (MAP). The vaccine currently on the market has some limitations including a severe injection site reaction and cross-reactivity with the tuberculosis test. Our goal is to identify candidates for a better Johne's vaccine.

Methods: Four MAP proteins were arrayed in 4 cocktails of 3 proteins each and then injected subcutaneously in 6-week-old Balb/c mice. MAP proteins were chosen based on IFN- γ responses and antigenicity. This was followed by a boost 3 weeks later and IP challenge with live MAP 2 weeks after the final boost. Serum and tissues were collected 3 months later for analysis. Splenocyte culture supernatants were harvested for measurement of cytokine secretion and cells were harvested for flow cytometric analyses.

Results: All MAP protein vaccine groups significantly reduced the recovery of live MAP from the ileum, while cocktails 2 and 3 showed a trend towards decreasing colonization in the liver. No significant differences were seen in mesenteric lymph node or spleen. Stimulation of splenocytes with a whole cell sonicate of MAP upregulated IFN- γ and IL-23 secretion in all treatment groups, regardless of vaccination. Interestingly, IL-4 was moderately downregulated for vaccinates compared to control infected mice. An increase in total CD25 expression was noted for 3 of the 4 protein vaccine groups upon stimulation of splenocytes with MAP, with this effect becoming more significant within CD4CD25 and CD8CD25 subpopulations.

Conclusions: Vaccination with cocktails of MAP proteins affected the host immune response after MAP challenge and decreased the amount of organisms in some tissues. Further analysis of these and other MAP proteins are still needed.

074P

Infection of B cells by *Brucella abortus*.

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Purpose: *Brucella abortus*, etiological agent of human brucellosis, infect and replicate into antigen presenting cells, such as macrophages and dendritic cells, and non-phagocytic cells as well. Although it has been demonstrated the interaction of this bacteria with B lymphocytes, its ability to entry and replicate into B cells is not demonstrated. In this work the capability of *B. abortus* to infect human or murine B cells was evaluated.

Methods: B cells of the cell line NALM6-R (human) or A20 (mice) were incubated with a suspension of *B. abortus* 2308. Infected B cells were examined by transmission electronic microscopy and CD69, CD40, CD80 and CD86 markers were determined by flow cytometry.

Results: Infected B cells showed the formation of phyllopodium near the site of contact with the bacteria. *Brucella* was internalized into one-layer membrane vesicles, and 24 h post-infection the bacteria was killed. When murine B cells were infected with *B. abortus* 2308 it was observed down-regulation of CD69 activation marker, CD40, CD80 and CD86 coestimulatory molecules and MHC-II in membrane surface.

Conclusions: These results suggest that *B. abortus* is internalized into B cells, probably through macropinocytosis, but it appears not to survive within. However, it could be a source of *Brucella* antigens to be processed and presented to T cells.

075P

Monocytes: The precursors of inflammatory dendritic cells in *Staphylococcus aureus* infection.

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Monocytes (MO) originate from myeloid precursors and circulate in the blood. They are considered precursors for tissue macrophages and dendritic cells (DC). The objective of this study was to determine the role of MO and their secreted cytokines in the process of DC differentiation during *S. aureus* (SA) infection. We hypothesized that following uptake of SA, MO secrete granulocyte-macrophage colony stimulating factor (GM-CSF), CCL2, and TNF- α resulting in autocrine stimulation and subsequent differentiation into DC. This study used bead purified CD14⁺ peripheral blood MO from bovines and SA strain RN6390B. SA uptake by MO was assessed by flow cytometry after infection with FITC labeled SA at multiplicity of infection (MOI) 10 and 25. As the MOI increased, the uptake of SA by MO also increased significantly, indicated by an increase in the percentage of FITC⁺ve MO. Real time PCR indicated a significant increase in the gene expression of TNF- α and GM-CSF after 24 and 48 h incubations. Presence of TNF- α and GM-CSF protein in supernatants of stimulated MO were confirmed by western blot. ELISA results showed that SA stimulation significantly increased CCL2 in culture supernatants compared to unstimulated MO. To determine the differentiation capacity of SA stimulated MO to DC, MO were infected with SA (MOI 10) for 2 h. After 2 h, cells were washed and fresh MO media without any exogenous cytokines was added and half of the media replaced every 3 days, and unstimulated MO were used as positive and negative controls, respectively. Phenotypic analysis of SA stimulated MO after 7 days of culture confirmed a distinct DC morphology as indicated by greater intensity of expression of CD11b, CD11c (b2 integrins), and MHC II and lower level of expression of monocyte marker CD14. Both morphology and receptor expression of SA stimulated MO were similar to positive controls. Results suggest that TNF- α and GM-CSF promotes MO differentiation to DC in SA infection. TNF- α is known to activate CCL2 and may induce b2 integrin expression on SA differentiated DC. In conclusion, SA stimulated MO differentiated into DC. Future experiments using TNF- α blocking antibody will confirm the role of TNF- α in SA stimulated DC differentiation.

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076P

Characterization of *Brucella abortus* infection of bovine monocyte-derived dendritic cells.

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Brucella abortus is a gram-negative facultative intracellular pathogen of cattle, and an important zoonosis in humans worldwide. Previous studies have shown that dendritic cells (DC) from humans and mice are highly permissive for *Brucella* survival and proliferation. Impairment of DC activation and maturation by *Brucella* infection has also been reported in these two species. This aim of this study was to characterize infection of bovine DC with *B. abortus*. Monocyte- derived DC (mdDC) were cultured from bovine peripheral blood mononuclear cells using the recombinant bovine cytokines IL-4 and GM-CSF. MdDC were infected with *B. abortus* strain 2308 at an MOI of 1 and 100. Bacteria were successfully killed by mdDC by 24 hours post infection (PI). Expression of IL-1b, IL-6, IL-10, IL-12p40, IFN, iNOS and TNF in *B. abortus* infected and LPS stimulated mdDC at 6 and 24 hours PI were evaluated using RT-PCR. At 6 hours PI all transcripts were increased over control cells and significantly less IL-10, IL-12p40, and IFN were expressed in mdDC infected with *B. abortus* compared to LPS stimulation. Flow cytometric evaluation of cultured mdDC showed approximately 70% of the cultured cells were DEC205⁺, MHC II⁺. Flow cytometric analysis of infected and LPS stimulated mdDC 24 hours PI showed expression of costimulatory molecules CD80 and CD86 was impaired in two of the three animals analyzed. MHC class II expression was equivocal between the groups. From these results we conclude that cultured bovine mdDC are not permissive for intracellular proliferation of *B. abortus*, and infected mdDC exhibit some signs of maturational and activational impairment compared to LPS stimulated DC.

077P

Proteomic analysis of Bovine Viral Diarrhea Virus infected monocytes.

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Purpose: Computational tools for high throughput biological data set analyses are designed to accelerate knowledge discovery in a rapid, accurate and efficient manner. However, biologists need to evaluate and apply appropriate tools for data analyses. Here we describe a combinatorial computational work flow that includes Gene Ontology and pathways analysis to proteomic datasets from a non-model organism. Pathogenesis of the disease caused by Bovine Viral Diarrhea Viruses (BVDV) in cattle is complex and involves persistent latent infection and immune suppression with a non-cytopathic (ncp) biotype during early gestation, followed by an acute infection by a cytopathic (cp) biotype. The molecular mechanisms that underscore the immune suppression in cattle caused by BVDV are not well understood.

Methods: Using comparative proteomics, we evaluated the effect of cp and ncp BVDV in bovine monocytes to determine their role in viral immune suppression and uncontrolled inflammation. Proteins were isolated by differential detergent fractionation and identified by 2D-LC ESI MS/MS. We carried out Gene Ontology (GO) based modeling using AgBase computational tools. Pathway analysis was carried out using Ingenuity Pathways Analysis (IPA). Results: Functional analysis of these proteins using the GO showed multiple under- and over-represented GO functions in molecular function, biological process and cellular component between the two BVDV biotypes. Analysis of the top immunological pathways affected by BVDV infection revealed that some pathways are affected only by one BVDV biotype but not the other one.

Conclusion: This combinatorial approach identified biotype-related differences in significant biological functions and pathways that could explain the observed biological differences.

078P

Ethyl pyruvate diminishes the endotoxin-induced inflammatory response of bovine mammary endothelial cells.

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Purpose: Despite improved dairy herd management techniques, coliform mastitis remains a devastating disease to the dairy industry. Furthermore, the endotoxin-induced inflammatory response during coliform mastitis is difficult to control with the currently available therapeutics. Endothelial cells are among the first cell type to be engaged in the inflammatory response and can modulate the severity of inflammation by producing pro-inflammatory mediators upon endotoxin exposure. Ethyl pyruvate, an ethyl ester of pyruvic acid, can ameliorate endotoxin-induced inflammatory responses by inhibiting the production of pro-inflammatory mediators in several in vitro and in vivo endotoxemia models. The objective of this study was to determine the effect of ethyl pyruvate on the production of vascular pro-inflammatory mediators that are associated with the pathogenesis of coliform mastitis.

Methods: The ability of ethyl pyruvate to reduce the expression of pro-inflammatory mediators was evaluated in cultured bovine mammary endothelial cells (BMEC) stimulated with endotoxin.

Results: Treatment of endotoxin-stimulated BMEC with ethyl pyruvate significantly reduced IL-6, IL-8, and intercellular adhesion molecule 1 gene expression as well as gene expression of eicosanoid-producing enzymes, including cyclooxygenase 2 and 15-lipoxygenase 1.

Conclusions: This is the first time that the effect of ethyl pyruvate was evaluated in an in vitro BMEC model of coliform mastitis. The ability of ethyl pyruvate to effectively inhibit the expression of potent vascular pro-inflammatory mediators, combined with the fact that ethyl pyruvate is safe for human consumption, may make ethyl pyruvate a highly attractive candidate as a therapeutic in ameliorating the severe pathogenesis associated with coliform mastitis.

079P

Selenoenzyme status affects eicosanoid biosynthesis in macrophages

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Purpose: Selenium (Se) is an essential micronutrient in the mammalian diet and its deficiency can lead to oxidative stress and immune dysfunction. Se functions as an antioxidant through the activity of selenoenzymes. Specific selenoenzymes can modify eicosanoid biosynthetic pathways by reducing fatty acid hydroperoxides and altering the activity of enzymes involved in the arachidonic acid cascade (i.e. cyclooxygenases (COX) and lipoxygenases (LOX)). It was established that both macrophages and oxidative stress play key roles in the progression of certain human diseases including atherosclerosis and cardiovascular disease; however, the relationship between selenoenzyme activity and eicosanoid biosynthesis in macrophages is currently unclear. Therefore, this study addresses the hypothesis that reduced selenoenzyme activity, such as that during oxidative stress, modifies the expression and activity of enzymes involved in eicosanoid biosynthesis.

Methods: Using both an *in vitro* RAW 264.7 Se-deficient model and an *in vivo* selenoprotein conditional knockout mouse model, we showed that significant decreases in selenoenzyme status resulted in accumulation of ROS and oxidative stress.

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Results & Conclusions: Significant increases in gene and protein expression were observed for several enzymes involved in eicosanoid biosynthesis including COX2, 5LOX and 15LOX. Studies are currently underway to profile the changes in lipid mediator biosynthesis as a consequence of altered COX and LOX expression. Further research is warranted to better elucidate the role selenoenzymes play in macrophage-derived eicosanoid biosynthesis and how these can alter the progression of atherosclerosis.

080P

Activation of porcine small intestinal epithelial IPEC-1 cells with bacteria-associated toll-like receptor ligands.

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Intestinal normal flora and enteric pathogens present many bacteria-associated molecular patterns to intestinal epithelial cells and these cells effectively respond to these bacterial ligands and mediate effective innate immune responses; however, immune responses of porcine intestinal epithelial cells to various bacterial ligands have not been studied in sufficient detail. The goal of this study was to define the changes in the expression of genes involved in intestinal innate immunity to bacteria-associated molecular patterns in pigs. We stimulated porcine small intestinal epithelial IPEC-1 cells with lipopolysaccharide (LPS), peptidoglycan (PGN), flagellin (FLA), CpG oligonucleotide (ODN) and GpC ODN for 3 and 24 hrs. Total RNA was extracted from cell lysates at each time-point and used to prepare cDNA using reverse transcriptase reagents. The gene expressions of various toll-like receptors (TLRs), NOD proteins, antimicrobial peptides, cytokines, and chemokines were quantified using real time RT-PCR relative to cyclophilin. The expression of TLRs and NOD proteins did not change upon stimulation with LPS, FLA, CpG and GpC at 3 hrs; however, PGN increased TLR-1, -2 and -6 expression at 3 hrs. LPS decreased TLR-4, -5, -6 and NOD-1; PGN decreased TLR-8 and -10; FLA decreased TLR-4, -8 and -10; CpG decreased TLR-4 and MD-2; and GpC decreased TLR-6 expression at 24 hrs. LPS stimulation induced increase in IL-1 alpha and CCL-20 expression at 3 hrs and increased IL-1 alpha and decreased IL-10 and MCP-1 expression at 24 hrs. PGN increased IL-1beta and osteopontin (OPN) expression at 3 hrs; and increased IL-1 beta and decreased IL-10 expression at 24 hrs. FLA increased IL-1beta, CCL-20, OPN and MIF expression at 3hrs; and increased IL-1beta and decreased IL-10 expression at 24 hrs. CpG induced increase in IL-12p40, IL-18 and MIF expression at 3 hrs; and increased MCP-1 and decreased IL-10 at 24 hrs compared to normal cells; however, no significant changes were induced by CpG when compared to GpC. These findings will help in understanding the basic biology of porcine intestinal epithelial cells and for developing effective preventive or therapeutic measures for enteric infections in pigs.

081P

Comparative proteomic analysis of protein modulation in bovine lung tissue during experimentally induced Mannheimia haemolytica pneumonia.

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Acute pulmonary infections in cattle are a continued focus in veterinary research due largely to staggering economic losses, and concerns regarding prophylactic antimicrobial use and the emergence of resistant strains of bacteria. Knowledge of protein modulation in healthy versus diseased bovine lung tissue could expand current knowledge of the mechanisms involved in the pathogenesis of bovine lung disorders, and aid in the identification of candidates for use as predictive or diagnostic biomarkers. The objective of the current study was to profile changes in proteins extracted from bovine lung tissues collected from cattle following in vivo challenge with Mannheimia haemolytica (M. haemolytica) using 2-dimensional gel electrophoresis (2D-GE). Proteins were extracted from lung tissue collected from 6 steers, 2 of which served as uninfected controls, and 4 that were infected with M. haemolytica. Proteins were excised from 2D-GE gels, digested with trypsin, and identified by peptide sequencing using matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) post source decay (PSD). Proteins that were modulated in diseased lung tissue when compared to healthy samples included: actin B (cytoplasmic), heat shock protein β -1, calmodulin, albumin, apolipoprotein A-1, annexin A2, annexin A4, serotransferrin, and cathelicidin-1, a member of the cathelicidin family of cationic antimicrobial proteins (AMP). The results indicate that examination of protein modulation in tissues during acute infections could aid in the identification of both biomarkers of disease as well as targets for novel veterinary therapies, as AMPs are currently being investigated for their therapeutic potential due to their specificity and broad-spectrum activity against a variety of bacterial pathogens.

082P

Equine hematopoietic progenitor stem cells are enriched in lineage-negative populations of cord blood mononuclear cells.

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Purpose: There are no available stem cell markers for the horse. The study of equine hematopoietic progenitor stem cells (HSCs) requires initial purification from the cell population of interest. The objective of this study was to enrich the numbers of HSCs obtained from cord blood mononuclear cell (MNC) populations using a negative selection column purification technique.

Methods: Cord blood MNCs were purified over density gradient and labeled with a panleukocyte (F6B) marker directly conjugated to PE. Labeled cord blood MNCs were then applied to an anti-PE microbead column. Cells obtained as the flow through and cells retained on the column were then seeded into colony assays. A semi-solid methylcellulose media containing growth factors for human HSCs was used with the addition of recombinant equine IL6, equine GM-CSF, feline stem cell factor and equine PBMC-conditioned media.

Results: Mean colony counts (mean = 11, range: 7-15) at 10 days were 10-fold higher in the F6B- fraction, than the F6B+ fraction (mean = 0.83, range: 0-2). Flow cytometric analysis of F6B+ and F6B- cell populations revealed that while the vast majority of T cells and monocytes had been removed, a significant population of B cells remained in the F6B- population. Cord blood MNCs were then labeled with both F6BPE and CD21PE and run over the anti-PE microbead column. Flow cytometric analysis of the F6B-CD21- fraction confirmed a largely lineage negative cell population. Mean colony counts at 10 days were 100-fold higher in this lineage negative population (mean = 98, range: 60-180) compared to unsorted cord blood MNCs (mean = 0.33, range: 0-1).

Conclusions: Equine hematopoietic progenitor stem cells can be enriched using negative selection from cord blood MNCs.

083P

Characterization of equine humoral antibody response to the nonstructural proteins of equine arteritis virus

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Purpose: Equine arteritis virus (EAV) is the causative agent of equine viral arteritis (EVA), a respiratory and reproductive disease that occurs in many equine populations throughout the world. EAV is an enveloped virus with a single-stranded, positive-sense RNA genome of approximately 12.7 kb. The genomic RNA comprises nine known open reading frames (ORFs). ORFs 1a and 1b encode two replicase polyproteins (pp1a and pp1ab) that are post-translationally processed by three ORF1a-encoded proteinases (nsp1, 2, and 4) to yield at least 13 non-structural proteins (nsp 1-12, including nsp7 α and 7 β). These proteins are expressed in EAV infected cells but the equine immune response to these proteins has not been studied. Therefore, the primary purpose of this study was to evaluate the humoral immune response of horses to each of the nsps encoded by ORFs 1a and 1b of the viral genome.

Methods: Individual nsp coding regions were cloned and expressed in both bacterial and mammalian expression systems. The authenticity of these recombinant proteins was confirmed by either protein specific rabbit antisera, mouse anti-His or anti-FLAG-tagged antibodies in immunofluorescence and Western immunoblotting assays. To characterize the humoral immune response to EAV nsps, the equine serum samples from horses that were seropositive to EAV were reacted with the recombinant proteins in immunoprecipitation assays. Subsequently, protein-antibody complexes were subjected to Western immunoblotting analysis with either individual nsp specific rabbit antiserum or mouse anti-His or anti-FLAG-tagged antibodies.

Results: The data showed that nsp2, nsp5 and nsp12 were the most immunogenic proteins among all nsps.

Conclusions: The data suggested that the nsps of EAV are immunogenic and stimulate antibody response in horses. Of the 13 nsps, nsp2, nsp5 and nsp12 appear to be the most immunogenic and these proteins may be suitable for the development of new serological assays. Information from this study will assist ongoing efforts to develop improved methods for the serologic diagnosis of EAV infection in horses.

084P

Humoral immune response to *Saprolegnia parasitica* in rainbow trout (*Oncorhynchus mykiss*).

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Purpose: *Saprolegnia parasitica* is an opportunistic pathogen that invades fishes, spreading across their epidermis as a cotton-like film. Because of this, *Saprolegnia* infections are often called "cotton mould". Infections caused by *Saprolegnia* mainly afflict fishes cultured under stress attributable to excessive manipulation, malnutrition, overpopulation and other concomitant infectious diseases. Here, we report the humoral immune response to *S. parasitica* in sera from rainbow trout.

Methods: *S. parasitica* was isolated directly from lesions and water-bodies of a trout hatchery near to Mexico City. The antigen was obtained from mycelium lysed by a French press, and the presence of antibodies anti-*Saprolegnia* was determined by ELISA.

Results: We evaluated serum samples from 28 *Saprolegnia*-infected fishes and 73 healthy fishes. Healthy fishes showed higher antibody titers as compared with infected trouts.

Conclusions: These results suggest a probable relationship between antibodies and protection against infections caused by *Saprolegnia* sp.

085P

Comparison of humoral and cellular immune responses to inactivated swine influenza virus vaccine in weaned pigs.

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Purpose: To evaluate and compare humoral and cellular immune responses to inactivated swine influenza virus (SIV) vaccine

Methods: Fifty 3-week-old weaned pigs from a herd free of SIV and PRRSV were randomly divided into the non-vaccinated control group and vaccinated group containing 25 pigs each. Pigs were vaccinated intramuscularly twice with adjuvanted UV-inactivated A/SW/MN/02011/08 (MN/08) H1N1 SIV vaccine at 6 and 9 weeks of age. Whole blood samples for multi-parameter flow cytometry (MP-FCM) and serum samples for hemagglutination inhibition (HI) assay were collected at 23 and 28 days after the second vaccination respectively. A standard HI assay and MP-FCM were performed against UV-inactivated homologous MN/08 and heterologous pandemic A/CA/04/2009 H1N1 (CA/09) viruses.

Results: While the HI assay detected humoral responses only to the MN/08 virus, the MP-FCM detected strong cellular responses against the MN/08 delta-cluster virus and lower but significant heterologous responses to the CA/09 gamma-cluster virus, especially in the CD4+CD8+ T cell subset.

Conclusions: The cellular heterologous responses to UV-inactivated gamma-cluster virus by MP-FCM suggested that the assay was sensitive and potentially detected a wider range of antigens than what is detected by the HI assay. The MP-FCM also identified the responses of multiple parameters in different T cell subsets. Overall, the adjuvanted UV-inactivated A/SW/MN/02011/08 H1N1 SIV vaccine stimulated both humoral and cellular immune responses including the CD4-CD8+ T cell subset.

086P

Pro-inflammatory and pro-apoptotic responses of TNF- α stimulated bovine mammary endothelial cells

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Purpose: Coliform mastitis may be severe in fresh cows due to enhanced expression of pro-inflammatory cytokines that contribute to disease pathogenesis. Tumor necrosis factor- α is implicated with the severity of coliform mastitis by provoking inflammatory responses in affected tissues. The endothelium is an integral organ in regulating inflammatory responses and loss of endothelial integrity may be fatal. Studies in humans suggest that endothelial cell apoptosis may be a consequence of TNF- α exposure and contributes to the development of sepsis, however, its impact on bovine mammary endothelial cells is unknown. We sought to determine the effects of TNF- α on primary bovine mammary endothelial cells.

Methods: Primary bovine mammary endothelial cell monolayers were stimulated with 10 ng/mL rbTNF- α . Gene expression was evaluated by qRT-PCR. Promega Glo caspase 3/7 & 8 were used to measure caspase activity. Cell viability was determined by Promega cell viability assay and flow cytometry.

Results: Stimulation of endothelial cells resulted in significant gene upregulation of toll-like receptor 4, interleukin-6 and -8, and intercellular adhesion molecule-1 and vascular cellular adhesion molecule-1 in a time-dependent manner. Caspase-3 mRNA expression increased significantly following TNF- α stimulation. Caspase-3/7 and -8 activity revealed an increasing trend towards 24 hr post-stimulation. Cell viability decreased slightly following stimulation. Flow cytometry showed an increasing trend in apoptosis towards 24 hr.

Conclusion: Results from our study support an early pro-inflammatory response in BMEC following TNF- α stimulation and suggest endothelial apoptosis may be an additional subsequent response to TNF- α stimulation. Endothelial apoptosis secondary to cytokine stimulation may contribute to the pathophysiology of severe coliform mastitis and warrants further investigation.

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087P

Immune and genetic control of swine responses to Porcine Reproductive and Respiratory Syndrome Virus Infection.

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Porcine reproductive and respiratory syndrome virus (PRRSV) is the most important infectious disease threat to pig production worldwide. Current vaccines are only partially protective. Thus it is essential to determine factors involved in protective immune responses against PRRS and the role of host genetics in resistance to PRRSV infection. To probe more deeply into the effects of PRRS on pig health and related growth effects the PRRS Host Genetics Consortium (PHGC) was established. Using a nursery pig model to assess pig resistance/susceptibility to primary PRRSV infection 8 groups of 200 crossbred pigs from high health farms were infected with PRRSV and followed for 42 days post infection (dpi). Blood serum and Tempus (RNA) samples were collected at 0, 4, 7, 10, 14, 21, 28, 35 and 42 dpi, and weekly weights recorded. Genomic DNA from all PHGC1-6 pigs were prepared and genotyped with the Porcine SNP60 SNPchip for current genome wide association studies (GWAS). Results have affirmed that all pigs become PRRSV infected with peak viremia from 4-21 dpi. Bivariate statistical analyses of viral load and weight data have identified PHGC pigs in different virus/weight categories. Sera and RNA are now being analyzed for immune factors involved in recovery from infection, including speed and levels of immune cytokine expression. These studies should identify biomarkers that distinguish PRRS resistant/maximal growth pigs from PRRS susceptible/reduced growth pigs. Overall, the PHGC project will enable researchers to verify important genotypes and phenotypes that predict resistance/susceptibility to PRRSV infection. The PHGC is funded by the US National Pork Board, USDA ARS, USDA NIFA PRRS CAP and functional genomic grants, NRSP8 Swine Genome and Bioinformatics Coordinators, and private companies.

088P

Antibody responses to BVDV in persistently infected (PI) cattle vaccinated with a combination of commercial and experimental BVDV vaccines.

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Purpose: To determine if BVDV PI animals can develop antibody after multiple vaccinations with BVDV vaccines.

Method: Four BVDV persistently infected (PI) cattle, 6 months to 4 years of age, were repeatedly vaccinated with a combination of infectious and noninfectious adjuvanted commercial vaccines containing Type I and II serotypes of BVDV. The cattle were also vaccinated with their own persisting BVDV, as well as the BVDV persistently infecting the other cattle. The persistently infecting BVDV isolates were given as adjuvanted noninfectious, as well as infectious, virus vaccines. Cattle were vaccinated both intramuscularly and subcutaneously multiple times over a 6 month period. Serum and whole blood was collected at various times after vaccination. Assays included serum virus neutralization, antigen capture enzyme-linked immunosorbent assay, and polymerase chain reaction.

Results: High titers of neutralizing antibody developed to both Type I and Type II viruses, irrespective of the persisting type of BVDV, after varying periods of time. However, no neutralizing antibody developed to the persisting BVDV isolates. Also, the animals remained persistently infected, as determined by antigen capture ELISA tests and PCR. Unfortunately, the study was terminated when the cattle died of rumen acidosis.

Conclusion: Although high antibody titers were produced to Type I and II viruses, no neutralizing antibody developed to the persisting strains of BVDV, thus the cattle remained persistently infected. In future studies, animals will continue to be revaccinated in an attempt to break "immunologic tolerance" to the persisting BVDV. To date we have results for twelve BVDV PI cattle that have cleared their BVDV. These cattle were persistently infected from 2 to 5 years prior to the clearance of persisting BVDV. These cattle were shown to remain free of virus and some had calves that were also PI free. These same cattle previously gave birth to PI calves.

089P

A comparative field study of BVDV Type 1 and 2, and BHV-1 immunologic memory to infectious (MLV) and non-infectious (killed) vaccines in dairy cows.

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Purpose: To evaluate the ability of conventional commercial vaccines to stimulate memory B cells (antibody production) in cows previously immunized with infectious (MLV) vaccines.

Method: Two Holstein dairy farms were chosen and the study was conducted on both farms simultaneously. The smaller herd, farm 1, is very well managed and consists of 2250 animals total. The larger herd, farm 2 is well managed for its size and consists of 3500 animals total. Both are semi-open herds and BVDV persistently infected animals have not been reported on either farm. Five vaccines were used: three infectious (MLV) vaccines - Ft. Dodge (Pyramid ®), Intervet/SP (Vista ®) and Pfizer (Bovishield ®); and two non-infectious (killed) vaccines - Ft. Dodge (Triangle9+BDVII®) and Pfizer (CattlemasterGold5FP®). Blood samples were taken and cows were vaccinated according to manufactures recommendations with randomly selected vaccine. Eighty-one Holstein cows from farm 1 ranged from 27 to 40 days in milk (DIM) at the onset of the study. The majority of cows were greater than 30 DIM. Sixty Holstein cows from farm 2 ranged between 200 to 210 days carrying calf (DCC) at the onset of the study. Blood was collected at onset of study and approximately one month post vaccination. Serum virus neutralization assay was performed to determine antibody titers of the animals to BVDV Type 1 and 2, and BHV-1.

Results: All products performed similar in their ability to stimulate increased antibody production during both stages of lactation, regardless of the vaccine. Cows 200 DCC were approximately 34% more immunologically responsive to boosting with BHV-1 than fresh cows.

Conclusion: These results suggest there is a significant increase in the ability to enhance antibody response to BHV-1 in late term pregnant cows as compared to cows 27 to 40 days after freshening. The reason for this difference and its potential significance will be discussed.

090P

Comparison of the efficacy of single or booster vaccination of Bison with *Brucella Abortus* Strain RB51.

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Free-ranging bison in Yellowstone National Park have an estimated 50% seropositivity for brucellosis. Effective strategies to address the endemic *Brucella* infection in bison will require development of safe and efficacious vaccines and vaccine delivery methods for free-ranging wildlife. In this study, we evaluated the immune responses after single vaccination with *B. abortus* strain RB51 using parenteral or dart delivery, and compared it to immune

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responses of bison parenterally booster vaccinated approximately 15 months after initial RB51 vaccination. Single vaccinates (dart or parenteral) had greater ($P<0.05$) antibody, proliferative, and interferon responses after inoculation when compared to controls. After booster vaccination, bison had greater ($P<0.05$) antibody responses but did not demonstrate significant differences ($P>0.05$) in proliferative or interferon responses as compared to single vaccinates. When challenged with a virulent *B. abortus* strain during pregnancy, none of the booster vaccinates aborted after challenge. In comparison, 83% of nonvaccinates, 50% of single parenteral and 57% of single dart vaccinates aborted. Although localization in the mammary gland was high for all treatments, the reduction in recovery of the challenge strain from uterine samples obtained from booster vaccinates suggests that this vaccination strategy would reduce lateral transmission of brucellosis. Although greater cellular immune responses were not detected, our data suggests that RB51 booster vaccination of bison enhances efficacy as compared to single inoculations.

091P

Comparison of immune responses in aged horses given commercially available live or inactivated equine influenza (EI) vaccines.

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Age-related decline in immune responses in horses is associated with reduced responses to vaccination and susceptibility to equine influenza (EI) infection. The aim of the present study was to compare the immune responses of aged horses given 2 doses of commercially available live or inactivated influenza vaccine. Twenty-six old horses (≥ 20 yrs) were included in this study, all of which had prior exposure to EI vaccination or infection since all had pre-existing haemagglutinin inhibition (HI) antibody titers. Treatment groups were stratified based on HI titers and body condition scores: Group 1 ($n=7$) inactivated EIV vaccine (OH/03 & KY/95 & Newmarket/93), Group 2 ($n=7$) inactivated EIV vaccine (KY/97), Group 3 ($n=7$) live recombinant vectored EIV vaccine (KY/94 & Newmarket/93) and Group 4 ($n=6$) non-vaccinate controls. Serum samples were collected prior to vaccination (day 0) and on days 7 & 14 post vaccination. Fourteen days (day 28) after the first vaccination, all horses received a second vaccination, and serum samples were collected on days 35 and 42. Antibody responses were measured by HI and single radial haemolysis (SRH) against the homologous virus for each vaccine and against a heterologous virus (KY/02). There was no significant difference when comparing HI and SRH titers against homologous and heterologous viruses prior to and post vaccination for any group. Prior to vaccination, there was no significant difference in HI or SRH antibody titers between treatment groups. Post vaccination, there was a significant increase in both HI and SRH titers for all three vaccine groups (day 0 vs. day 7, $P<0.05$) difference in HI or SRH antibody titers when comparing day 7 vs day 14 results for all vaccine groups. Both inactivated vaccines induced a significantly ($P<0.05$) higher antibody responses (HI and SRH) when compared to the recombinant vaccine. Administration of a second vaccine dose did not increase the antibody responses in any of the vaccine groups.

092P

Absence of glycan moieties in GP3 & GP5 of a PRRSV field isolate enhances its susceptibility to antibody neutralization and its ability to elicit neutralizing antibody response

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Passive transfer of PRRSV-specific neutralizing antibodies (NABs) to pigs prior to infection results in complete protection, demonstrating the important role of NABs in protective immunity. However, pigs infected with PRRSV typically develop a weak and delayed NAB response which results in ineffective resolution of the infection. During our studies on sub-typing of PRRSV isolates by cross-neutralization, we found a type-II PRRSV field isolate, designated as O1, which is able to elicit an atypically rapid and robust NAB response in infected pigs. In addition, O1 is extremely susceptible to antibody neutralization. Analysis of structural genes of O1 revealed that the virus naturally lacks 2 glycosylation sites in its envelope glycoproteins: one in GP3 at position 131 and the other in GP5 at position 51, both of which are normally present in other PRRSV strains. To determine the influence of the alteration of glycosylation patterns on the distinct neutralization phenotypes of O1 isolate, a chimeric virus was generated by replacing the structural genes of FL12 cDNA infectious clone with the counterparts of O1. The resulting chimeric virus, designated as FLO1, maintains the same characteristics of its parent O1, demonstrating that the NAB phenotype segregates with the structural genes of PRRSV. Using FLO1 as a backbone, glycosylation sites were re-introduced into the glycan missing positions in GP3 and GP5 separately or in combination, by site-directed mutagenesis. Re-introduction of the glycosylation site in either GP3 or GP5 significantly reduced the susceptibility of the virus to antibody neutralization in vitro and its ability to elicit NAB production in vivo. Interestingly, synergistic effect was observed when the glycosylation sites were re-introduced simultaneously in GP3 and GP5. Although the absence of these two glycosylation sites occurs naturally, O1 virus quickly regains those glycosylation sites throughout the process of replication in vivo, suggesting a strong selective pressure at those sites. Collectively, our data demonstrates for the first time the involvement of a glycan moiety located in GP3 in interference with the development of NAB to PRRSV.

093P

Potential T-cell epitopes present in nonstructural proteins 9 and 10 of type-II Porcine Reproductive and Respiratory Syndrome Virus eliciting IFN- γ response.

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PRRSV live attenuated vaccines currently in use are effective at conferring protection against homologous strains, but less protective against heterologous strains. It is already known that cell-mediated immunity (CMI) plays an important role in establishing protective immunity by PRRSV vaccination. Likewise, it has been speculated that in the case of PRRSV, the animal with a good virus-specific T cell response would bear a milder infection, consequently leading to reduced mortality and less spread of the virus among swine population. A detailed characterization of the conserved T-cell epitopes contained in the PRRSV genome is of cardinal importance towards understanding and possibly modulating the PRRSV-specific CMI. Non-structural viral proteins should contain major conserved domains which are potential T cell epitopes and hence need serious consideration. Our aim in these experiments was to find T-cell epitopes in nonstructural protein 9 (nsp9, that helps in genome replication and transcription) and 10 (nsp10, which encodes for helicase function). Two libraries of synthetic heptadeca peptides, with a 9 amino acid overlap, encompassing the entire sequence of either Nsp9 ($n=78$, encompassing 646 aa) or Nsp10 ($n=54$, encompassing 441 aa) were analyzed to determine their T-cell epitopes using T-cell proliferation assay and IFN- γ ELISPOT assay. These functional assays were performed using peripheral blood mononuclear cells isolated from pigs that had been either infected ($n=8$) with PRRSV type II strain FL-12 or non-infected ($n=8$). Four out of 78 nsp9 peptides and 2/54 nsp10 peptides were found to induce T-cell

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proliferation. Of these, only 2 peptides of nsp9 and 2 peptides of nsp10 were confirmed in their immunogenicity using ELISPOT IFN- γ assay. In addition, upon sequence analysis of 34 North American PRRSV strains isolated worldwide (source: NCBI) using ClustalW, it was observed that these epitopes were highly conserved. These immunodominant epitopes can be used in the formulation of immunogens to provide broad cross-protection against diverse PRRSV strains.

094P

Use of attenuated *Erysipelothrix rhusiopathiae* strains as vectors for *in vivo*-delivery of porcine IL-18 for immunomodulation.

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Purpose: Interleukin-18 (IL-18), which was originally identified as an interferon- γ inducing factor, has been shown to play an important role in control of infections. In this study, attenuated *Erysipelothrix rhusiopathiae* strains expressing porcine IL-18 were evaluated for their potential immunomodulatory capacities in mice and pigs.

Methods: Attenuated acapsular YS-1 and Koganei 65-0.15, the live vaccine strain used in Japan, were engineered to produce porcine IL-18 as a chimeric protein with SpaA, a cell surface protective antigen of *E. rhusiopathiae*, and designated YS-1/IL-18 and KO/IL-18, respectively.

Results: Recombinant IL-18 was detected in the samples from bacterial cell surface preparation and culture supernatant, suggesting that IL-18 is not only exposed on the cell surface but also released in the supernatants. In BALB/c mice intraperitoneally (i.p.) inoculated with YS-1/IL-18, significantly higher concentration of INF- γ was produced by concanavalin A-stimulated splenocytes, compared to the mice inoculated with the parental strain YS-1. In YS-1/IL-18-inoculated mice, phagocytosis of *Salmonella* Typhimurium by peritoneal macrophages was significantly enhanced, and after oral inoculation with *Salmonella* Typhimurium, growth inhibition of the bacteria in spleen, mesenteric lymph nodes and Peyer's patches were observed, compared to the mice inoculated with YS-1. In pigs inoculated orally with KO/IL-18, higher amount of IL-18 was detected in serum and bronchoalveolar lavage fluid (BALF), and the levels of IgA and IgG antibodies against SpaA in serum and BALF were higher, compared to the pigs orally inoculated with Koganei 65-0.15, suggesting that the strain has adjuvant activity.

Conclusions: These findings provide evidence that *E. rhusiopathiae* is a promising vector for *in vivo*-delivery of host cytokines and also suggest its potential utilization for activation of immune responses to control infections.

095P

IL-1beta cooperates with TGF-beta to induce invasion and stem cell formation in Gliomas.

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Purpose: The epithelial-mesenchymal transition (EMT) is an embryonic process that becomes latent in most normal adult tissues, but is often activated during wound healing and cancer progression. The link between EMT and stem cells holds a number of implications in the cancer progression. The EMT process enables cancer cells to acquire mesenchymal traits necessary for dissemination from a primary tumor, and stem cell traits essential for initiation of secondary tumor.

Methods: The sphere assay has commonly been used to identify stem cells, which can proliferate as spheres under serum-free condition supplemented with epidermal growth factor (EGF) and basic fibroblast growth factor (bFGF). Studies have shown that TGF-beta is a major inducer of EMT and the EMT cells induced by TGF-beta have the ability to form spheres. Interleukin-1beta (IL-1beta) is a pleiotropic cytokine and aberrant production IL-1beta is tightly linked to tumor generation and poor prognosis in many types of cancers, suggesting that IL-1beta may contribute to cancer stem cell biology. To test the hypothesis, we used an intestinal cell line IEC18 to study the effects of IL-1beta on stem cell production by applying sphere assay.

Results: We found that the control cells without IL-1beta treatment formed very few small spheres, suggesting that the cell line contain very few stem cells. Addition of IL-1beta or TGF-beta resulted in significant increase of the number and size of spheres. The combination of IL-1beta and TGF-beta induced the most number and largest size of spheres.

Conclusions: These results indicate that IL-1beta functions similarly to TGF-beta to induce sphere formation and growth, and the combination of IL-1beta and TGF-beta produces the synergetic effects on sphere proliferation. We will further characterize the properties of these induced spheres. Overall, our results suggest that inflammatory cytokine IL-1beta induces stem cell self-renewal and it synergizes with TGF-beta to induce stem cell formation.

096P

IL-1beta cooperates with TGF-beta to induce invasion and stem cell formation in Gliomas.

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Purpose: Gliomas are the most common primary brain tumors in adults. Recent studies show that gliomas contain a small population of cells with potent tumorigenesis and stem cell characteristics. These glioma stem cells (GSCs) are resistance to radiotherapy and chemotherapy, and are considered responsible for the initiation and recurrence of gliomas. Therefore, targeting GSCs is a more effective therapeutic strategy against gliomas. Transforming growth factor beta (TGF-beta) and interleukin-1beta (IL-1beta) are both highly active in high grade gliomas and their elevated activities have been associated with poor prognosis in glioma patients.

Method: In this study, we determined their contribution to GSC development using human glioma cell line LN229 and serum-free condition. The serum-free medium was commonly used to isolate neural stem cells (NSCs) and GSCs, which proliferate as spheres in this condition. Interestingly, LN229 cells cannot form spheres in serum-free media, suggesting that these cells contain very few stem cells.

Results: We found that IL-1beta or TGF-beta alone induced only a few of sphere formation. Surprisingly, combination of IL-1beta and TGF-beta induced significant more spheres, indicating synergetic effects of IL-1beta and TGF-beta on induction of spherical cells. The induced spheres by IL-1beta and TGF-beta showed significantly increased invasion and drug resistance, and correspondingly up-regulated expression of invasive genes, drug resistant genes and stem cell genes, compared to the control cells without cytokine treatment or the cells treated with IL-beta or TGF-beta alone.

Conclusions: These results indicate that IL-1beta cooperates with TGF-beta to induce invasion, drug resistance and GSC formation. Our finding may explain why highly active TGF-beta and IL-1beta in high grade glioma are associated with poor prognosis in patients. Thus, our study shed lights on the contribution of TGF-beta and IL-1beta to cancer stem cell (CSC) biology. A better understanding of how CSCs develop is likely to lead to novel therapeutic strategies for the successful treatment of patients.

RESPIRATORY DISEASES POSTERS

097P

Bactericidal effect of tracheal antimicrobial peptide against bovine respiratory pathogens.

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Purpose: To determine if tracheal antimicrobial peptide (TAP) kills the bacterial pathogens that cause respiratory disease in cattle. TAP was the first beta-defensin discovered and has bactericidal activity against many bacteria, yet the effectiveness against bovine pathogens has not been characterized. Since some pathogens appear to be resistant to some innate immune defences, presumably as an evolutionary adaptation to allow survival in the host, future work with TAP depends on whether this antimicrobial peptide is specifically effective against pathogens of relevance to cattle.

Methods: The following bacterial isolates were obtained from the lung of cattle with pneumonia: *Mannheimia haemolytica* (n=8), *Histophilus somni* (n=3), *Pasteurella multocida* (n=4); in addition, a single enteric isolate of *Escherichia coli* was used. Antibacterial activity of TAP was measured using an agar-based radial diffusion assay, and using a broth dilution method in which bacteria were exposed to various concentrations of TAP for 2 hours before measuring the surviving number of colony forming units.

Results: In the radial diffusion assay, the addition of 0.25 to 2.0 micrograms of TAP resulted in dose-dependent inhibition of growth of all bacteria tested. Using 2.0 ug of TAP, the diameters of inhibition were 10.8±0.4 mm for *M. haemolytica*, 8.0±0 mm for *H. somni*, 9.7±0.25 mm for *P. multocida*, and 11.0 mm for *E. coli*. The dilution method showed similar dose-dependent bactericidal activity, and the overall average minimum inhibitory concentration was 6.1 ug/ml.

Conclusions: Tracheal antimicrobial peptide has bactericidal activity against bovine disease-associated isolates of *M. haemolytica*, *H. somni* and *P. multocida*, and differences in sensitivity among isolates of the same pathogen were not identified.

098P

Isolation of *Histophilus somni* from the nasal exudate of a clinically healthy adult goat.

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H. somni was isolated for the first time, as causing encephalitis in bovines and, subsequently, in cases of meningoencephalitis and thromboembolic meningoencephalomyelitis. Other signs of the septicemic form are myocarditis, otitis, mastitis, conjunctivitis, and polyarthritis.

In ovines, its participation has been reported in orchitis, epididimitis, mastitis, abortion, synovitis, meningoencephalitis, septicemia, and pneumonia. There is only one report in goats of an isolation from the vagina, but no information is available on the presence of this microorganism in their nasal cavity. The objective of the present study was to isolate *H. somni* from samples of nasal exudate of clinically healthy goats, from the Mixteca Region of Puebla State, Mexico.

We evaluated nasal exudates from 42 goats of the Mixteca Region in the State of Puebla, Mexico. After 4 days of incubation, we isolated a strain that was identified according to its phenotypic characteristics and by means of a species-specific PCR, as well as by sequencing the amplification product. The PCR amplified a 407 pb fragment and sequencing revealed 100% homology with *H. somni* 129PT. The nucleotides sequence was deposited in the GenBank under Access Number HM032735. The herds in which the present study was performed were constituted exclusively of goats, which eliminates de interspecies spreading factor. The presence of *H. somni* opens the question whether it is a microorganism forming part of the normal flora or should it be considered an opportunistic pathogen and, therefore, a potential problem in goats. This is the first isolation worldwide of *H. somni* from samples of nasal exudate in a clinically healthy goat.

099P

The role of the white-tailed deer immune response in the presence of BVDV infection.

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Purpose: Bovine Diarrheal Virus is a viral disease that is a major concern within the cattle industry. In the recent decade a rising concern is focusing on other domesticated and wild ruminants that have been shown to be susceptible and spread BVDV. White-tailed deer (*Odocoileus virginianus*) have been documented to be persistently infected with BVDV causing a high risk of transmission of BVDV from deer to cattle. It is still unclear the significance of the BVD in white-tailed deer but causes a possible biosecurity threat to cattle producers.

Methods: In this study white-tailed deer were infected with a BVD strain isolated from a persistently infected deer found in South Dakota. On post infection (P.I.) days 3, 6, 9, 11, 13, 17, 19, blood samples were taken and immune cell surface markers were analyzed using flow cytometry to understand the population of immune cells in the presence of BVDV. White blood count (WBC) was done to correlate the immune cell populations to the classical BVDV leucopenia seen in the white-tailed deer infected with the BVDV deer isolate.

Results: By day 9 there was a decrease expression of CD14; day 11 an increase CD4 positive T-cells was observed while the WBC show BVD caused leucopenia. Virus isolation (VI) showed that the virus was being actively shed by infected white-tailed deer.

Conclusions: The results from this study show the significance that BVDV actively replicates causing a viral immune response and sheds in white-tailed deer which could be transmitted to cattle. This study helps to better understand the role of the white-tailed deer's immune response in the presence of BVDV infection. This knowledge will help scientists to make decisions on controlling BVDV in wild ruminant species.

100P

Antigenic variations associated with poor performance of direct fluorescent antibody test to detect bovine viral diarrhoea virus (BVDV) II antigen in fresh tissues.

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Antigenic variations associated with failure of immunohistochemistry and ELISA to detect BVDV field isolates has been previously reported. However, the impact of the antigenic diversity on the performance of commercially available fluorescent antibodies against BVDV has not been thoroughly studied. In this study, three different methods were used to detect BVDV in the tissue specimens of two animals with clinical signs compatible with BVDV infection.

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The first animal was an adult beef breed cow with multifocal shallow erosions and ulcers were appreciated in the oral cavity predominantly along the soft palate, the tongue, and the esophagus. Immunohistochemistry (IHC) revealed the presence of BVDV antigen in the epithelial cells lining tubuloacinar mucous and mixed glands of the oral mucosa. BVDV was isolated from the tissue pool and genotyped as BVDV II by a real time reverse transcriptase-polymerase chain reaction (RRT-PCR). However, FA with proper positive and negative controls failed to detect BVDV antigen in these tissues. The second animal was a feedlot calf that had died following an episode of bloody diarrhea. BVDV was isolated and genotyped as BVDV II. Direct FA again failed to detect BVDV antigen in the tissue specimen of this animal. Cross neutralization test revealed antigenic differences between the two isolates and between each isolate and the reference strain (125c). The sequences of the 5' UTR and E2 region of the isolates were determined. Phylogenetic analysis showed that these two isolates belonged to different clusters. In conclusion, the results of this study indicated that genetic and antigenic diversity among BVDV isolates affected the performance of direct FA test on fresh tissue specimens.

101P

Adenosine-5'-triphosphate release by lipopolysaccharide and interleukin-1 stimulated bovine lung epithelial cells

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Purpose: Mannheimia haemolytica, one of the agents associated with bovine respiratory disease complex, can cause severe lung pathology including the leakage of vascular products into the airways and alveoli. Previous work by this lab has demonstrated that bovine lung endothelial and epithelial cells undergo dramatic permeability increases when exposed to adenosine-5'-triphosphate (ATP).

Methods: A chemiluminescence assay for ATP was used to determine whether ATP levels were elevated in bronchoalveolar lavage (BAL) samples from calves experimentally infected with M. haemolytica. In addition, cultured bovine lung epithelial (BPE) cells were stimulated with lipopolysaccharide (LPS), lipoteichoic acid (LTA), interleukin-1 (IL-1), and zymosan activated plasma (ZAP) to determine whether these products might stimulate the release of extracellular ATP by these cells.

Results: Calves experimentally infected with M. haemolytica had an approximately 2-fold higher level of ATP in their BAL samples compared to control. BPE cells treated with several concentrations of LPS and IL-1 had significant increases in ATP release as compared to time-matched controls. This increase appeared to be a result of active ATP secretion by the cells, as cell viability was similar between treated and non-treated cells. Neither ZAP nor LTA induced any ATP release by the cells.

Conclusions: ATP levels are elevated in lung secretions from calves infected with M. haemolytica. In addition, lung epithelial cells can actively release ATP when exposed to LPS or IL-1.

102P

Generation of human telomerase reverse transcriptase-immortalized porcine monocyte/macrophage cell lines.

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Porcine reproductive and respiratory syndrome virus (PRRSV) shows highly restricted cell tropism and targets subpopulations of differentiated macrophages such as porcine alveolar macrophages (PAMs) in the natural host. Although primary PAMs would be ideal for *in vitro* virus infection, they are not only difficult and expensive for establishment but cannot be frozen reliably for long-term storage and use. In addition, African green monkey kidney derived Marc-145 cells are often used for virus propagation. However, concerns have been raised regarding a possible modification of specific epitopes associated with virus neutralization because of distinct virus entry in PAMs and Marc-145 cells. In order to overcome these problems, the present study was aimed to generate immortalized porcine monocyte/macrophage cell lines and to evaluate their potential for PRRSV production. Thus, primary PAMs were stably transfected with the human telomerase reverse transcriptase (hTERT) cDNA by a retrovirus vector so that constitutive expression of the hTERT protein allows cells to proliferate indefinitely. The newly established PAM cell lines were shown to express robust levels of the native CD163 receptor in their surface. Subsequently, we demonstrated that these PAM cell lines are fully permissive for PRRSV infection. Our results suggest that the hTERT-immortalized PAM cell lines can enable us to facilitate the continued use of PAMs for virus isolation and production and to provide a tool for viral pathogenesis and immune function studies.

103P

Preliminary study of PRRSV inactivated vaccine efficacy in vaccinated piglets.

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Porcine reproductive and respiratory syndrome virus (PRRSV) is an economically important pathogen and characterized by respiratory distress in piglets and reproductive failure in sows. Although modified live-attenuated vaccines (MLVs) and many inactivated vaccines were developed but PRRSV remains difficult to control. MLV strongly induce virus-neutralizing antibody but have with the risk of reverting to virulence. Inactivated vaccine is safety and flexibility towards emerging virus strains. But all of two types vaccine limited to protect pigs against a heterologous challenge with PRRSV. Nauwynck et al. presented that vaccination with binary ethylenimine(BEI)-inactivated PRRS vaccine induced virus-specific antibodies and strongly primed the virus-neutralizing antibody response. In this study, we prepared experimental PRRSV inactivated vaccines, based on optimized BEI inactivation procedure instead of formaldehyde. Vaccine contained 4 PRRSV strains that are 1 European type and 3 North American types which are prevalent in South Korea in order to get the wide range of protection. Sixteen piglets were vaccinated twice. Vaccinated piglet distributed in 4 groups for challenge with each strains. 15 additional piglets remained unvaccinated and served as positive(n=12)and negative(n=3) controls. After vaccination, piglets did not show any clinical signs or adverse reactions. 2 weeks after challenge, blood and organ samples were collected for check viremia, serological change and gross lesions to evaluate vaccine efficacy. Vaccination reduced the severity of microscopic lesions in lung. Whereas positive controls showed moderate mild interstitial pneumonia and enlarged lymph nodes.

104P

Genetic diversity of Porcine Reproductive and Respiratory Syndrome Virus in Korea.

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This study was performed to detect PRRSVs, to differentiate their genotypes and to assess their genetic diversity in field samples collected from pig farms associated with wasting and/or respiratory syndrome during the last five years. To detect PRRSVs using RT-PCR, tissue samples were collected from 786 pig farms associated with clinical signs during 2005~2009. To assess genetic diversity of Korean PRRSVs, phylogenetic trees were constructed with complete open reading frames (ORF) 5 of the type 1 and type 2 PRRS. Different sequences of the Korean PRRSV isolates were compared with those of various PRRSV isolates from other countries. 643 farms (81.8%) of the pig farms examined were positive for PRRSV, of which 57.2% accounted for PRRSV type 1 and 70.2% accounted for PRRSV type 2. Furthermore, 37.5% of the farms positive for PRRSV, showed the coexistence of two genotypes. ORF5 sequences of type 1 and type 2 PRRSV analyzed were 117 and 198, respectively. Analyzed Korean type 1 and type 2 PRRSV sequences showed a homology of 81.6~100% and of 81.4~100.0%, respectively. In phylogenetic analysis, Korean type 1 PRRSVs were classified into three clusters based on ORF5 genetically distinct from LV strain, while Korean type 2 PRRSVs were classified into four clusters based on ORF5. Analyzed Korean PRRSV isolates were distributed throughout the clusters independently of the isolation time and geographical origin. In putative amino acid sequence analysis, all of the B-cell epitopes were highly conserved among Korean type 1 PRRSVs. Especially, Korean type 1 PRRSVs contained highly conserved two or three potential N-linked glycosylation sites and one neutralization epitope in GP5. In the case of Korean type 2 PRRSVs, three B-cell epitopes in GP5 had highly variable sequences. Especially, two to five potential N-linked glycosylation sites in GP5 were found to be distributed throughout type 2 viruses. The results also demonstrated that mixed infection with type 1 and type 2 PRRSVs and genetic diverse isolates are very common in Korean pig farms.

105P

Detection of Porcine Reproductive and Respiratory Syndrome Virus using a DNA microarray chip.

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The coexistence of two genotype-PRRSV in pig herds emphasizes the need of the development of rapid and reliable methods of virus detection and genotyping. The microarray can be useful to overcome the several risks and limits of molecular diagnosis of veterinary fields because it gives more genetic information than RT-PCR. PRRSV microarray contains 10 unique probes designed against highly conserved genotype-specific ORF6 and ORF7 regions of PRRSV genomes. Through multiplex RT-PCR amplification strategy and hybridization on microarray chip, PRRSV detection and genotyping were done as a single assay. VR-2332 and LV strains were correctly detected and differentiated using the developed microarray. The specificity of the array was excellent, as no viral agents relevant for differential diagnosis yielded a positive reaction. The limit of detection of the microarray to PRRSVs was exhibited detection by $10^{-1.0}$ TCID₅₀/ml of PRRSV VR-2332. The amplified PCR products were diluted at low concentrations (2ng/ul) and the array was repeated four times and all exhibited the correct detection of PRRSV. A panel of 149 field samples which included single or mixed genotype-infections and non-infections were used for validation. The accuracy of PRRSV microarray to differentiate genotype was compared with RT-PCR and nucleotide sequencing. It was verified by PRRSV detection in 149 field samples, out of which 121 and 136 samples were positive for PRRSV ORF6 and ORF7 in RT-PCR, respectively. In ORF7-targeted array, 71 were positive for type 2 infections, 61 for mixed-genotype infections, and 4 for type 1 infections. With ORF6-targeted array, 100 were positive for type 2 infections and 21 for mixed-genotype infections. The PRRSV microarray showed high sensitivity with 92.6% for ORF6 and 100% for ORF7, respectively and the specificity of the array was all 100% for two regions. In microarray analysis for the ORF6- and ORF7-targeted probes, the results showed 5 and 4 signal patterns including negative reactions, respectively. The results demonstrate that the PRRSV microarray complements current methods and can accelerate the diagnosis and genotyping of PRRSV.

106P

Sensitivity and specificity of ELISA tests for serological diagnosis of PRRSV Type 1.

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Purpose: Porcine reproductive and respiratory syndrome virus (PRRSV) strains belong to the two genotypes (Type 1 and 2) which differ antigenically. There is evidence of the restricted serological cross reactivity also between different genetic subtypes within Type 1 but it is unknown whether it impacts the sensitivity or specificity of the current ELISA diagnostic tests.

Methods: To address this problem 275 serum samples were obtained from 3 Polish, 4 Belarusian and 1 Ukrainian farms, where different genotypes and subtypes circulated as it was determined by PCR and DNA sequencing. Additionally, samples from farms free from PRRSV were used. All the samples were tested twice with HerdChek 2XR and 3X (IDEXX), Ingezim PRRS Universal, Europa and America (Ingenasa) and the in house test produced in our laboratory.

Results: All ELISAs detected PRRSV specific antibodies in sera from all farms where PRRSV infection was present. Comparison of the serological profiles of one farm (sera from 4, 6, 8, 10 and 12 weeks old pigs) obtained with the different tests showed that Ingezim PRRS Universal is significantly less sensitive than the remaining tests in detection of maternal antibodies in pigs at 4-8 weeks of age. While the IDEXX kits and NVRI in house tests detected PRRSV specific antibodies in 16-17 samples of 24 from pigs of such age, Ingenasa kit detected antibodies in only 5 of them. The analysis of the specificity proved it to be lowest in case of the HerdChek 2XR test. It gave false positive results with majority of 13 sera from single rector pigs as it was found before. Remaining tests gave no (NVRI in house, HerdChek X3) or up to three (Ingezim kits) false positive results with these sera.

Conclusions: All of the universal (or specific for PRRSV Type 1) ELISA kits are based on the recombinant capsid protein of the subtype 1 of PRRSV Type 1 so the observed variation between them can be attributed to difference in the antigen processing or ELISA reagents formulations. Analysis of more commercially available ELISA kits is being performed and the complete results will be presented at the symposium.

Acknowledgement: The study was supported by the grant from Polish Ministry of Science and Higher Education, no. N N308 265136.

107P

Serological efficacy of Circumvent™ PCV in pigs from Korea.

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Purpose: Several commercial PCV2 vaccines have been widely used in the Korean swine industry. However, the effects of the vaccines on post-weaning multi-systemic wasting syndrome (PMWS) have never been unequivocally demonstrated. The purpose of this study was to evaluate the efficaciousness of vaccination against PCV2 has the efficacy of Circumvent™ PCV with ELISA and real-time PCR in Korean field conditions.

Methods: Vaccinated animals in each farm were inoculated with commercial PCV2 vaccine at 3 and 6 weeks of age. Blood samples of individual animals were collected before the first and second vaccinations and in each stage of finishing phase. Serological analysis in serum was performed by commercial

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enzyme-linked immunosorbent assay (ELISA) for antibody level and real time polymerase chain reaction (real time PCR) for the quantification of viral load. Student t-test or Wilcoxon Mann-Whitney test was used to assess differences with regard to parameters of viremia and serology.

Results: Serum antibody level increased in placebo-treated animals after booster vaccination without infectious antibodies. The Sero-positive rate showed remarkable differences between the vaccinated group and the placebo-treated group at 10-16 weeks of age. Moreover, in the vaccinated group, the antibody level tended to decrease at 3 weeks after the first vaccination, but was increased until 16 weeks of age by the booster shot applied at 6 weeks of age. Moreover, the percentage of animals that were definitely or equivocally sero-positive was consistently maintained until slaughtered age. Conclusions: This study was the first attempt to evaluate a commercial vaccine against PCV2 in Korean fields. The vaccination regimen stimulated high antibody levels and reduced PCV2 viral loads, supporting the beneficial effects of vaccination against PCV2 in the control of PCV2 infection.

108P

Results of CicumventTM PCV in pigs on growth performance and mortality rate.

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Purpose: This field study was conducted to investigate the effect of vaccination against porcine circovirus type 2 (PCV2) in three commercial Korean farms experiencing porcine circovirus associated disease.

Methods: Pigs approximately 20-days-of-age were assigned to the group injected with a commercial two dose vaccine (vaccinated group) or placebo group injected with saline. Injections consisted of intramuscular injection at weaning and 3 weeks later. All pigs were weighed prior to the first and second vaccinations and in each stage of the finishing phase. Mortality rate was recorded. The results provide evidence that vaccination against PCV2 is effective in improving growth performance and reducing mortality rate in pigs raised on commercial Korean farm with a history of PCVAD, especially in the finishing phase.

Results: As seen average daily weight gain, it was considered to be an objective measurable parameter to determine the severity of PCVAD and the effects of vaccination in a large number of animals. Vaccinated groups had significantly improved growth performances more than placebo-treated group in either or both Entry into the finishing phase or/and midpoint of the finishing phase. The total of mortality rate from vaccinated group was significantly lower during the finishing phase, compared with placebo-treated group.

Conclusions: The results provide evidence that vaccination against PCV2 is effective in improving growth performance and reducing mortality rate in pigs raised on commercial Korean farm with a history of PCVAD, especially in the finishing phase.

109P

Prevalence of antibodies to avian influenza viruses and risk factors for exposure in Thai free-grazing duck flocks.

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Purpose: The presence of free-grazing duck flocks in Thailand has been repeatedly identified as a potential risk factor for highly pathogenic avian influenza H5N1 outbreaks in poultry. In a unique, extensive livestock management system, duck farmers move their flocks of 1,000-10,000 ducks from one post-harvest rice field to another, where they feed on residual rice, insects and snails. Spatial analysis has shown that the density of free-grazing duck flocks and rice cropping intensity are factors which are statistically associated with outbreaks of highly pathogenic avian influenza H5N1. Little is known, however, about the transmission events that occur within and among flocks, the prevalence and incidence of infection, and the virus subtypes to which the ducks are exposed. The goals of this study were to estimate the proportion of sampled flocks with avian influenza antibodies, identify potential risk factors for infection, and use these findings to develop recommendations for improved avian influenza surveillance of free-grazing duck populations. Methods: In this study, we conducted a cross-sectional serologic survey of free-grazing duck flocks, with corresponding flock owner interviews, in Suphanburi Province, Thailand. A random sample of registered free-grazing duck flocks was selected for participation in the study. In addition to being a registered flock, the inclusion criteria included that the owner had taken the current flock of ducks to graze openly on a rice field at least once prior to sampling. Flock owners were interviewed regarding flock characteristics, as well as grazing, contact and movement history. Blood was drawn from thirty ducks in each flock and analyzed by blocking enzyme-linked immunosorbent assay (ELISA), using the FlockCheck Avian Influenza MultiS-Screen Ab Test Kit (IDEXX Laboratories, Westbrook ME). The percentage of seropositive flocks was determined using a sample/negative (S/N) cutoff determined to be most appropriate for duck samples ($S/N < 0.6$ = positive, with a suspect-positive zone at $0.6 < S/N < 0.7$). Flock parameters were analyzed for association with ELISA positivity. Data collection was completed between July and September, 2010.

VECTOR-BORNE AND PARASITIC DISEASES POSTERS

110P

Serological and molecular detection of equine piroplasms in Korea

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Purpose: Equine piroplasms include two tick-borne protozoan parasites, the exclusively intraerythrocytic *Babesia caballi* and the intraerythrocytic and intralymphocytic *Theileria equi*. While Korea is recognized internationally as an endemic region of equine piroplasms, there is no report on equine piroplasms. This study was initiated to detect *B. caballi* and *T. equi* in blood of horses reared in Korea.

Methods: Blood samples of 184 horses (*Equus caballus*) reared in multiple areas in Korea during 2007 to 2010 were analyzed by serological and molecular methods. For serological detection, horse sera were assessed for the presence of antibodies against *B. caballi* and *T. equi*, by using cELISA kits, respectively (VMRD Inc., USA). For molecular detection, the DNA isolated from horse blood was amplified with primers derived from the conserved regions in the 18S rRNA of piroplasms. For specificity of the PCR product and differentiation between species, nested PCR and complete sequencing of the 18S RNA were done. Then, phylogenetic analysis was performed using MEGA (V.4.0).

Results: In serological analysis, 2 (1.1%) of 184 sera were positive for *T. equi* by cELISA. Whereas, all tested samples were negative for *B. caballi* by cELISA. In molecular analysis, 2 (1.1%) of 184 samples were positive for *T. equi* by PCR and sequencing. The samples (GG-7 and GG-14) positive for *T. equi* by cELISA were also positive by PCR. Thus, cELISA was shown to be as sensitive as PCR in this study. The phylogram of complete sequences of *T. equi* 18S rRNA showed high degree of identity (98.6-99.8%) with GenBank databases for *T. equi*. The sequences of *T. equi* GG-7 and GG-14 (accession nos. HM229407 and HM229408, respectively) were clustered together with *T. equi* isolates from Spain, Sudan and South Africa, which indicates the possibility of a close epidemiological link among these isolates.

Conclusions: To our knowledge, this is the first report to describe the serological and molecular detection and phylogenetic analysis on *T. equi* from horses in Korea. Since infection of *T. equi* exists in horses reared in Korea, further studies on hemoparasites and appropriate control programs need to be established.

VIRAL PATHOGENESIS POSTERS

112P

System for viral and mycoplasma contamination control of veterinary preparations and row materials.

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Purpose: To develop a PCR-based assay for viral and mycoplasma contamination screening of biological row and biopreparations according to modern international and Ukrainian requirements.

Methods: We designed primers and developed PCR-based protocols to screen row (cell cultures, sera, and media), productive viral seed, and ready-to-use products for the presence of DNA/RNA viruses and mycoplasma DNA. BVDV protocol was developed for 5'-UTR and Npro gene amplification. Additionally, we amplified rep gene fragment from porcine circovirus (PCV) DNA, as well as two sequences from the mycoplasma genome: a fragment of 23S rDNA and fragment of 16S. The protocol employed 42 cycles utilizing a primer annealing temperature between 58 and 64°C. Detected viral contaminants were genotyped by partial sequencing of Npro gene of BVDV and rep gene of PCV2.

Results: Triplex PCR for both mycoplasma and viral nucleic acid detection was successfully developed. Methods were validated in accordance with OIE requirements, and results indicated high sensitivity (approaching 98-100%) and analytic sensitivity (0.1 lg of BVDV and PCV, 150-200 CFU of Mycoplasmas), specificity (94-97%), repeatability, and reproducibility. When compared with single target PCR, and with regard to the practical application of this newly developed technique, a higher level of BVDV contamination was detected in blood used for cell culture sera: up to 12% samples were positive with multiplex, compared with 10% using a single target. Sequence analysis indicated that PCR amplification identified the presence of the BVDV 1a genotype. Mycoplasma contamination was detected in 2-12% samples tested. PCV contamination was detected in 2.5% of row samples (sera and cell cultures) of porcine origin. Sequence analysis of this contaminants identified 1a, 1b, and 2a PCV2 genotypes. Products positive for contamination were rejected and destroyed.

Conclusions: We developed a PCR approach for effective screening of bioproducts and row for the presence of BVDV, PCV, and mycoplasma. This diagnostic platform will be used as a National standard for detection of viral and mycoplasma row contamination.

113P

RNA interference of feline herpesvirus by synthetic siRNAs in corneal epithelial cells.

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Purpose: Feline herpesvirus 1 (FHV-1) is a DNA virus that causes upper respiratory tract infections in cats. Repeated viral recrudescence produces corneal disease and can potentially lead to blindness. We have shown previously that RNA interference can be used to dramatically reduce feline herpesvirus (FHV-1) replication in Crandell-Rees feline kidney (CRFK) cells by targeting essential viral genes, including the DNA polymerase and glycoprotein D. Further studies have been performed in CRFK cells to determine optimal combinations of siRNAs to produce the greatest reduction in virus replication. These experiments have been extended to primary feline corneal cells, an *in vitro* model more closely resembling the *in vivo* environment preferred by the virus. The objective of this study was to evaluate combinations of siRNAs in the corneal cells.

Methods: Various transfection agents were evaluated and conditions were optimized for this cell line to reduce toxicity within the cells, while maintaining ≥90% transfection efficiency. Interference efficacy was assessed by measuring amounts of glycoprotein D and DNA polymerase mRNA by real time RT-PCR in treated, untreated, and control samples and by determining viral protein expression on the surface of infected cells in each group by flow cytometry. Plaque assay was also performed to titer infectious virus in each sample.

Results: Six different combinations of siRNAs were tested, and each of these produced a significant reduction in virus replication compared to the control. The two most effective combinations produced 98%±1% and 97%±1% reductions in infectious virus. Treatment with these combinations resulted in reductions in FHV-1 DNA polymerase mRNA by approximately 80% and glycoprotein D mRNA by approximately 90% compared to the untreated control. FHV-1 proteins were also reduced on the surface of treated cells by approximately 60%.

Conclusions: RNA interference using combinations of siRNAs targeting FHV-1 mRNAs was successful in reducing replication of FHV-1 in feline corneal epithelial cells. Additional studies should be performed to evaluate uptake and toxicity of topically delivered siRNAs into feline corneal cells *in vivo*.

114P

Identification of a novel herpesvirus DNA sequence in cutaneous ulcer lesions found in a sudden death case of a fisher (*Martes pennanti*).

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Two captive born fisher males and females of two and a half years old were bought from a breeding farm in Minnesota, USA, and sent to the St-Félicien Zoo, Québec, Canada, on October 2nd 2008. Animals were in good health and after a quarantine period, they were housed in a natural enclosure. The fisher is a fur animal of the size of a domestic cat with a long tail and short legs classified in the *Mustelidae* family, his scientific name being *Martes pennanti*. On December 8th 2008, one fisher male was found dead with ulceration on the muzzle and plantar pads. At necropsy, no major finding was found except the ulceration located at all paws and foot pads, and the muzzle. In addition, no salient histopathological finding within the specimens examined could explain the death of the animal. Herpesviruses are well known to cause ulcers of the skin and mucosa to several mammals. Thus, a previously reported pan-herpesvirus nested-PCR assay, which is able to detect a broad spectrum of herpesvirus species, was conducted. An expected PCR product of 248 nucleotides (nt) was obtained with tissues collected around and from the ulcers lesions. Other tissues or pool of tissues (intestines and pool of lung, liver and kidney) were tested and they were all negative. Afterwards, the PCR amplicon obtained with the skin tissues was sequenced (GenBank accession number HM579931) and comparison by BLAST tools showed that the nearest identity was around 80% with the DPOL viral gene of a badger herpesvirus, which is classified in the *Herpesviridae* family within the subfamily *Gammaherpesvirinae*. The following acronym FiHV was given to this new herpesvirus. Virus isolation was attempted using MDCK (Madin-Darby canine kidney), CRFK (Crandell-Reese feline kidney) and ML (mink lung) cell lines and embryonated eggs. For all types of sample tested, no CPE was observed within all the cell lines used. Electron microscopy did not reveal the presence of virion in the cell culture lysates and supernatants, and in allantoic fluids of inoculated eggs. The involvement of FiHV with any diseases such as ulcers lesions has to be confirmed. Now, it is even more difficult to assume a relationship between the FiHV and the death of the animal.

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Transcript expression analysis in tracheobronchial lymph nodes of pseudorabies virus infected pigs.

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Purpose: This study addresses the critical relationship between Pseudorabies virus (PRV) and its host at a transcriptional level during the course of an infection.

Methods: RNA isolated from draining tracheobronchial lymph nodes (TBLN) specimens from 5-week old pigs clinically infected with a feral isolate of PRV (FS268) and uninfected were interrogated using Illumina Digital Gene Expression (DGE) Tag Profiling to better understand the physiopathology of infection and the immune response at a cellular level.

Results: Over 100 million digital gene expression tag sequences were observed, representing 4,064,189 unique 20-base sequences collected from TBLN at time points 1, 3, 6 and 14 days post-infection. Multidimensional statistical tests were applied to determine which changes in tag abundance were significant and tags were annotated with transcriptomic information.

Conclusions: The experimental results have been integrated with previous studies to develop a robust model of swine respiratory virus infection.

116P

The search of new ways of eradication of herpesvirus infections.

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Purpose: Herpesvirus infections widespread all over the world and are one of major medical and social problems. Herpesvirus I type, which are infect 90-99% of adult population of our planet, is most widespread. Herpesvirus I type cause also the row of diseases for domestic and wild animals.

For this reason the main goal of our researches was a search of new ways of fight against Herpesvirus I type, and more precisely different means, as chemical, so, phylogenous origin with the purpose of search of effective and safe preparations for prevention and chemotherapy of herpesvirus diseases.

Methods: test-model virus - herpesvirus type 1; continuous animal cell cultures: cell culture of versenised swine embryonic kidney (CCVSEK), BHK-21, Vero and CPT (cell culture of pig testicles). For investigation of anti-virus properties we used a complex of standard methods. The cytotoxicity of all testable preparations of in vitro system (cell culture) and sharp toxicness of preparations of in vivo system (laboratory mice of BALB/c line and guinea-pigs) was also investigational in accordance with standard methodologies. Results: Results of experimental researches of cytotoxic and antiviral action of preparations of a different origin and their different mixed are presented. We studied the row of new means and new, before not studied compositions of means, and indicate their anti-virus properties. All examined means significantly reduced the infection activity of virus strains. New antiviral means reduced infection activity to $1,51 \pm 0,08 - 2,1 \pm 0,1$ lg [CID50/ml], compositions of the means - to $2,4 \pm 0,06 - 4,6 \pm 0,21$ lg [CID50/ml]. But the highest antiviral activity among tested substances was shown by using new composition of means (to $5,54 \pm 0,04$ lg [CID50/ml]).

Conclusion: As a result of the conducted investigation which can become basis for the production of medicinal forms are found, effective and safe preparations for a chemotherapy at the herpesvirus infections of a 1 type.

117P

Antibody response of pigs to the E protein of Porcine Reproductive and Respiratory Syndrome Virus.

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The E protein, also known as 2b protein, is a non-glycosylated, minor structural protein of the Porcine Reproductive and Respiratory Syndrome virus (PRRSV). The E protein is encoded by the open reading frame 2b starting from the +6 nucleotide position in mRNA2 and it is embedded in the phospholipid bilayer of the virus. It consists of 73 and 70 amino acids in the North American and European strains of the PRRSV, respectively. With >70% amino acid sequence identity, this protein is one of the highly conserved structural proteins among all PRRSV strains sequenced so far. Topology prediction models suggest that the N-terminus of the E protein is exposed on the viral surface and the C-terminus is positioned inside the viral particle. A published study demonstrated that the pigs infected with PRRSV produce antibodies to the E protein. The goals of this study were to determine kinetics of E protein-specific antibody production during early stages of PRRSV infection, and to ascertain the domain specificity of the induced antibodies. Two 25 amino acids-long peptides that correspond to the N-terminus and C-terminus of the E protein were custom-synthesized and used in an enzyme-linked-immunosorbent assay (ELISA) as antigens to determine the presence of specific antibodies in the infected pigs. Analysis of serum samples collected from pigs experimentally infected with P129 strain of PRRSV showed that all the animals produced antibodies against the C-terminus, but not the N-terminus, peptide. The antibodies specific to the C-terminus peptide appeared in the serum as early as 10 days post-infection, peaked by day 14 and persisted until the termination of the experiment on day 21. Analysis of serum samples from commercial pigs indicated a positive correlation between the IDEXX ELISA results and the presence of the C-terminus peptide-specific antibodies. All PRRSV-infected pigs produced the C-terminus peptide-specific antibodies, irrespective of the infecting viral strain. These results suggest that the C-terminus peptide of the E protein is useful for the development of a broadly-reactive serological test for detection of PRRSV infection in pigs.

118P

Three amino acids of ORF5 are jointly responsible for virulence of porcine reproductive and respiratory syndrome virus.

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The virulence of porcine reproductive and respiratory syndrome virus (PRRSV) is considered to be multigenic because it is determined by the accumulation of various mutations throughout the genome. Recently our laboratory reported that two PRRSV genomic regions (non-structural protein 3 to 8 and ORF5) are important for virulence by generating a series of chimeric viruses between two distant (~90% homologous) PRRSVs (Infectious clone-derived highly virulent FL12 vs attenuated Prime Pac vaccine). Following those results, we focused in this study on just ORF5 using the FL12 and its closely related (~99% homologous) ATP Ingelvac vaccine strains, where FL12 and ATP differ only 3 amino acids (Val64Leu, Gly80Ser and Ile121Thr) in ORF5. To study the individual and combined contribution to virulence of these amino acids, single and triple mutants of ORF5 were generated by site-directed mutagenesis. While none of single/triple mutants showed any significant in vitro growth differences from wt FL12, ORF5 triple mutant-infected animals showed significantly lower viral loads than wt FL12 group in vivo, especially in early days post inoculation (dpi) periods, indicating a less virulent phenotype. The in vivo virulence phenotypes of single ORF5 mutants were variable although a change in amino acid position 64 seemed to contribute to

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most of the virulence. Upon analyzing the in vivo stability of ORF5 region, the amino acid at position 64 from some animals of the triple mutant group was found to be mutated back to wt FL12 at 21 dpi but not in the case of amino acid positions 80 and 121 as is also the case in ATP infected animals. Overall, this finding suggests that there are certain key amino acid residues in the ORF5 which contributes to PRRSV virulence. For the development of rationally attenuated new generation vaccines, other clusters of virulence markers should be further characterized.

119P

Interaction of PRRSV NSP1 β with the cellular poly (C)-binding proteins (PCBPs).

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Non-structural protein (NSP)-1 β is the second protein encoded by the PRRSV genome. NSP1 β cleaves itself off the replicase polyprotein (up stream of NSP2), via its cysteine protease activity. Over the years, investigations towards characterization of NSP1 β have shown many interesting features of this protein. Among the many functions described for NSP1 β , its role in regulation of viral RNA synthesis and its antagonism towards host innate immune system have been well characterized. To gain a deeper understanding of the regulatory nature of NSP1 β , we have performed co-immunoprecipitation coupled with mass spectrometry studies to find host cell proteins interacting with NSP1 β . In this study, the cellular poly (C)-binding proteins (PCBPs) - PCBP1 and PCBP2 were found to be two interaction partners of NSP1 β . Besides co-immunoprecipitation assay in PRRSV-infected MARC-145 cells, the interaction between PCBP1 and NSP1 β has been confirmed by in vitro GST-pull down assay. RNA appears to be part of this complex, as PCBPs are RNA binding proteins and treatment with RNase greatly reduces the NSP1 β -PCBP interaction. The PCBPs are major regulators of gene expression affecting mostly post-transcriptional events like pre-mRNA processing, mRNA stability and translation. Several viruses, especially members of Picornaviridae require PCBP1/2 to regulate viral transcription and translation. We speculate that the NSP1 β -PCBP interaction has a major role in regulation of PRRSV gene expression.

120P

Interaction of PRRSV Nsp1 α and protein inhibitor of activated STAT1 (PIAS1) mediates sumoylation of Nsp1 α .

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PRRSV is a cytoplasm-replicating virus but non-structural protein (Nsp1) has been shown to target to the nucleus, suggesting that Nsp1 may play an important role during infection via interaction with cellular nuclear proteins. Nsp1 is autocleaved into two products, Nsp1 and Nsp1, and in gene-transfected cells, Nsp1 localized to both cytoplasm and nucleus whereas Nsp1 was predominately found in the cytoplasm. To elucidate the nuclear role of Nsp1, yeast two hybrid screening was conducted using a cDNA library made from porcine alveolar macrophages. The protein inhibitor of activated STAT1 (PIAS1) was identified as a cellular protein interacting with PRRSV Nsp1. Nsp1 co-precipitated PIAS1 when co-expressed in cells, and the PIAS1-Nsp1 interaction was confirmed by GST pull-down assay. Porcine PIAS1 was 651 amino acids in length and contains SAP domain and RING-zinc finger domain which functions as a SUMO (small ubiquitin-like modifier) ligase and as a negative regulator for IFN signaling. Nsp1 was sumoylated when PIAS1 was over-expressed in PRRSV-infected MARC-145 cells as well as in HeLa cells expressing Nsp1, indicating that Nsp1 interacts with PIAS1 and acquires SUMO modification during infection. To determine the role of the newly identified zinc finger motif in Nsp1 for SUMO modification, a series of mutants were constructed to disrupt the zinc-finger configuration and examined for structure function relationship. The data show that the zinc-finger configuration plays a role for IFN regulation. Our study reveals important mechanisms of IFN regulation by PRRSV during infection.

121P

PRRSV Nsp1 beta subunit-based inhibition of interferon-beta production and signal transduction.

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Activation of type I interferon (IFN) during viral infection is essential for cellular resistance to virus infection. PRRSV infection however, induces a low level of IFN production and is often associated with persistent infection. Viral nonstructural protein 1 (Nsp1) is cleaved to Nsp1A and Nsp1B two subunits during virus infection and has been demonstrated a potent viral IFN antagonist. Nsp1A has been shown to possess a strong inhibitory activity on IFN production, and in the present study, the effects of Nsp1B on the IFN production pathway and IFN-induced signal transduction through the JAK-STAT pathway were investigated. Similar to Nsp1A, Nsp1B found to possess an IFN suppressive activity by targeting both IRF3- and NF-kB-dependent IFN production pathways. Nsp1B and Nsp1A repressed the IFN induction to different extents, and Nsp1B was less potent than Nsp1A. Inhibition of IFN by Nsp1B was not limited to the production pathway but also observed in the IFN-B induced JAK-STAT signaling pathway. Our results provide insights into how PRRSV escapes from host antiviral defense using Nsp1 as a viral antagonist.

122P

Efficacy of PRRSV vaccines against homologous and heterologous strain challenge

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Inoculation of gilts with an on-farm virulent PRRSV is a common practice for prevention of PRRS disease in the breeding herd. It is based in part on the perception that effective anti-PRRSV immunity requires exposure to the same virus that animals will be exposed to later in life. To examine the validity of this perception, we inoculated gilts with a highly virulent PRRSV, and challenged with the same virus (homologous) or 98.2% genetically similar virus (heterologous) about 210 days later, in late gestation. Key indicators of protection were abortions, weaned pigs, and piglet viremia at weaning. Aborted or dead-born litters were observed in 2/15 (heterologous), 2/14 (homologous) and 0/5 (challenge only) sows. The rate of healthy pigs weaned per total born alive was 82% (heterologous), 88% (homologous) and 0% (challenge only). Incidence of viremia at weaning was 27% (heterologous), 10% (homologous) and 95% (challenge only). Fever (temperature >39°C) was observed in 53% (heterologous) and 29% (homologous) of challenged sows, but peak temperature was not correlated with abortions. Neutralizing antibodies at challenge were absent or low, and not related to abortions. Levels of viremia in positive piglets at weaning were low in the majority of cases and there was no difference in antibody levels between viremic and nonviremic animals. There also was no difference in anti-PCV2 antibody levels. Total mortality rates [(total fetuses - total weaned)/total fetuses] were about the same in heterologous and homologous groups at 45% in contrast to 70% in the challenge only group. Negative control inoculation only mortality was 4%. In conclusion, virulent PRRSV inoculation provided substantial protection against reproductive PRRS and homologous and heterologous challenge models. Homologous protection resulted in a lower rate of PRRSV-positive piglets at weaning, but transmission of PRRSV to piglets was not prevented.

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Development of highly pathogenic Avian Influenza and Newcastle Disease molecular detection and differentiation techniques.

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Purpose: To develop PCR assays for detecting HPAIV and NDV, and to test methodologies according to international standards for HPAIV and NDV diagnostics, monitoring, and differentiation.

Methods: PCR primers and protocols were designed and optimized according to annealing temperature, mix composition, and thermal cycling kinetics. Protocol efficiency was analyzed in the context of efforts to monitor HPAIV and NDV in wildlife. The system for HPAI detection was based on classical PCR diagnostic principles, with amplification of a 560 base pair (bp) M-gene fragment for detection, and 487 bp and 580 bp HA-gene fragments for H5/H7 differentiation, respectively. Amplification temperature parameters were 57°C for M gene and 60°C for differentiation. NDV detection was based on amplification of 560 NP-gene and 345 bp F-gene fragments, with PCR carried out at 58°C and 62°C, respectively, using 2,5 and 3,8 mM Mg²⁺, respectively.

Results: Development of a platform for simultaneous detection of both viruses was based on the newly developed protocols and multiplex touch-down PCR. PCR protocols (single and multiplex) designed in the current experiments permitted identification of 6 novel HPAIV (H5N1) isolates and 18 NDV isolates, which were detected in poultry and wild birds, and were validated according to OIE requirements.

Conclusions: Effective protocols and techniques were developed in accordance with OIE recommendations for HPAIV-NDV diagnostics and differentiation, thereby improving upon the current system for monitoring, diagnostics, and viral identification of these diseases.

124P

Serological supervisions of avian influenza among wild birds in Ukraine in 2000 - 2009.

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Purpose: Influenza remains an unpredictable infection for animals, birds, and people. Occurrence of new strains and variants requires constant supervision and their careful studying. Because of Ukraine occupies a unique geographical position in the centre of Europe, and through its territory pass transcontinental migratory ways of wild birds from northern Asia and Europe to the Mediterranean, Africa and Southwest Asia, carrying out serological monitoring of influenza among wild birds was our purpose.

Methods: Presence of antibodies was defined in blood serum and egg yolks by the standard techniques. Preparation of yolks was conducted by own patented technique.

Results: The presence of antibodies to a influenza virus of subtypes H1, H2, H3, H5, H6, H8, H9, H10, H12, and at synantropic birds of 9 species antibodies to influenza H, H8 are revealed in 2000-2003 at migrating waterfowls of 4 species. In 2005-2006 in blood serum and egg yolks there were found out antibodies at Mallard, Ruddy Shelduck to H2, H6, in Yellow-legged Gull - H3, H4, H5, H7, Cormorant - H1, H5, H8, Slender-billed Gull and Gull-billed Tern - H2, Common Tern - H8, Mediterranean Gull - H2, H4, H7 H9. In egg yolks of Great Tit, Collared Flycatcher, Song Thrush Blackbird antibodies to influenza virus H1, H2, H4, H8 are found out. In 2007 the antibodies to influenza A virus H3 were found out at Grey Plover, Coot, to H5 at Coot, to H6 at Ruff, to H8 at Ruff and Avocet, to H9 at Ruff, Marsh Sandpiper, Dunlin, Ringed Plover, to H13 at Curlew Sandpiper, Mallard - to H5. In egg yolks of Black-headed Gull - to subtypes H5, H14 and Bluethroat - H5, H14

Conclusions: So, antibodies to 9 subtypes of influenza A virus were detected in different wild birds, that stimulate necessarily for wide monitoring work for AI in wildlife

125P

Identification of a recombinant porcine norovirus and a porcine sapovirus of a new genogroup in Korea.

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Norovirus (NoV) and sapovirus (SaV) identified in humans and pigs evidence relatively high mutation rates, as do most other RNA viruses. In this study, a total of three strains of NoV and 37 strains of SaV were detected in 567 porcine fecal samples, corresponding to prevalences of 0.5% and 6.5%, respectively. Phylogenetic analyses were conducted using amino acid sequences of the partial RNA-dependent RNA polymerase (RdRp) and complete capsid proteins of both viruses to determine their genotypes or genogroups. Two strains (DG32 and HW41) and one strain (DO35) of the three porcine NoVs were classified into genotype II-18 (GII-18) and GII-11, respectively. The amino acid and nucleotide sequence analyses showed that the DO35 strain should be a recombinant virus harboring the RdRp region derived from the SW43-like NoV strain and the capsid region originated from the SW918-like NoV strain. Phylogenetic analysis using the RdRp sequences of porcine SaVs revealed that a total of 26 strains selected from the 27 strains subjected to genogroup analyses were classified into genogroup III (GIII). The remaining strain (DO19) was not clustered with any of the previously classified SaV strains, thereby suggesting the advent of a new genogroup virus. Additional analyses with the amino acid sequence of the capsid and the nucleotide sequence of the RdRp and capsid junction region further supported the notion that the DO19 strain belonged to a novel genogroup. To the best of our knowledge, this is the first study to describe a recombinant strain of porcine NoV and a porcine SaV belonging to a novel genogroup in Korea.

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Fetal immunological effects and liver tolerance following persistent bovine viral diarrhea virus infection.

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Bovine viral diarrhea virus (BVDV) a member of the pestivirus group of the flavivirus family is associated with the largest number of viral respiratory and reproductive disease cases in cattle in the United States. Infection of a fetus during the first trimester of pregnancy leads to a condition called persistent infection (PI). Current BVDV PI control methods rely on vaccination programs that have moderate success. This study was designed to measure the specific immunological effects that occur in the innate immune response of the fetus following BVDV infection and investigate the role of liver tolerance in persistent infection. Kupffer cells (liver macrophages) from fetal livers of BVDV PI and control fetuses at 96 days of gestation (21 days post BVDV infection) were isolated and two macrophage immune functions, phagocytosis and antigen presentation, were compared to uninfected controls. Additionally, IHC analysis of fixed tissue was done to assess antigen distribution within the PI liver. There was an increase in the phagocytic activity of the Kupffer cells in BVDV infected fetuses as compared to control fetuses in freshly isolated cells. MHC I expression was increased in persistently infected

cultures as well. BVDV antigen was widely dispersed throughout the liver. Understanding the immunological mechanism of the fetal tolerance and characterization of the immunological effects could strengthen understanding and further develop a better vaccination strategy.

127P

Clinical characterization of Bovine Viral Diarrhea Virus from Korean indigenous calves.

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Purpose: Bovine viral diarrhea virus (BVDV) is the etiological agent of bovine viral diarrhea (BVD) and an economically important worldwide disease in the livestock industry. To investigate BVDV circulating in Gyeongbuk province which is the highest cattle population density in the Republic of Korea (ROK), we examined 473 diarrhea stools from clinically affected Korean indigenous calves in 13 cities of Gyeongbuk province.

Methods: Total RNA was extracted using Trizol from the diarrhea stools. RT-PCR was performed with Superscript™ One-Step RT-PCR System with Platinum Taq. Amplification and sequencing of 5'-UTR was performed using 324/326 primers. Phylogenetic tree based on the nucleotide alignments was constructed using the neighbor-joining (NJ) method. Bootstrap analysis was carried out using 1000 replications and the tree was visualized using Treeview.

Results: Of these, 75 cases tested positive for BVDV by RT-PCR. Phylogenetic analysis revealed that all our cases were classified as BVDV-2a. Our cases presented for this study from which BVDV-2a was isolated exhibited clinical signs of watery diarrhea (89%) and bloody diarrhea (11%).

Conclusions: Therefore, this result will be useful to understand epidemiology and pathogenesis of BVDV infection found in Gyeongbuk province. Also, the results suggest that a vaccine development and immunization strategies are recommended for the effective control of BVDV infection in the ROK.

ORAL ABSTRACTS

BACTERIAL PATHOGENESIS

001

Mastitis in selected areas in the Philippines: prevalence, etiology, and antibiotic sensitivity profile

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Purpose: To establish the extent of mastitis problem in local dairy farms and determine the common bacterial agents involved in these intramammary infections and their antibiotic sensitivity profiles, a cross-sectional study on mastitis was done.

Methods: This included detection of mastitis cases using California Mastitis Test (CMT) among backyard dairy herds in Laguna and Batangas, bacterial isolation and identification from identified mastitic cases by conventional isolation procedures and automated miniaturized identification using BBL Crystal ID™ identification kits, and in vitro antibiotic susceptibility profiling of each isolate recovered and identified using the Kirby-Bauer method.

Results: A total 48 (49.5%) of the 97 backyard dairy cows tested were found to have at least one mastitic udder. From the 88 milk samples collected from these mastitic udders, a total of 132 bacterial isolates were recovered and identified. The two most common organisms found were *Staphylococcus aureus* (31.06%) and *Escherichia coli* (12.88%). Overall, 62 of the 82 (75.6%) isolates were found resistant to at least one of the following antibiotics: penicillin, ampicillin, streptomycin, amoxicillin, cefixime, oxytetracycline, TMPs, amoxi-clav, erythromycin, and cefaclor. Almost all *Staphylococcus aureus* isolates were found to be susceptible to all antibiotics except cefixime. Of the antibiotics tested, oxytetracycline was found to have the widest efficacy with 75 (91.5%) of the isolates being found susceptible and 7 (8.5%) as intermediately susceptible. On the other hand, cefixime was found to be the least efficacious, with 39 out of 56 (69.6%) of the isolates being found as resistant. Several isolates, particularly *Bacillus cereus*, coliforms and *Pseudomonas* species were also found to have multiple resistance to different antibiotics.

Conclusions: Although antibiotic therapy may be a useful therapeutic tool, the importance of good management practices and education of farmers to control mastitis should nonetheless be equally emphasized.

002

Identification of *Staphylococcus aureus* genes highly expressed during bovine mastitis: A unique opportunity for vaccine development.

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Bovine mastitis is a disease of major concern for the dairy industry and *S. aureus* intramammary infection (IMI) is one of the leading causes for this pathology. Efficacious treatment is still unavailable and this is partly due to the complexity of *S. aureus* IMI and a lack of knowledge about the virulence factors involved. In order to identify virulence factors, eight cows were experimentally infected with three *S. aureus* strains and the infection was followed for 21 days. Bacteria were isolated after milking and gene expression was followed and quantified using DNA arrays. Among the strongly expressed genes detected in the three strains and different cows during IMI, eleven genes were of particular interest as they had never been detected in any previous surrogate or animal models of infection. Expression of several of these genes during IMI was confirmed by qPCR. Two genes, one for which expression was induced by milk components and another for which expression was strictly induced by intramammary growth, were further evaluated as vaccine candidates. First, knock-out mutants were prepared and their capacity to infect cows was evaluated over a 21-day period. During IMI, the mutants were found to be attenuated as the concentration of viable bacteria was significantly reduced for the mutants as compared to the parental strain. Recombinant proteins derived from those 2 genes were produced and their immunogenicity was tested in animals. Both proteins were immunogenic in mice and cows. In conclusion, genes expressed during bovine IMI were discovered and their use in a vaccine is highly promising.

003

Bovine mammary endothelial cell inflammatory responses after in vitro *Streptococcus uberis* challenge.

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Purpose: Routine use of dry cow therapy has reduced the incidence of intramammary infections caused by contagious agents. At the same time, environmental pathogens, such as *Streptococcus uberis*, have emerged as the major causes of mastitis in heifers and low somatic cell count herds. Prolonged and uncontrolled *S. uberis*-induced mastitis often results in severe tissue damage and significant milk production losses. Inflammatory responses during intramammary infections are dependent on interactions between the pathogen, vasculature and immune cells.

Methods: The objective of this study was to characterize the inflammatory response of bovine mammary endothelial cells (BMEC) following in vitro culture with *S. uberis*. BMEC were allowed to grow to confluence prior to the addition of *S. uberis* to a transwell membrane at a final bacteria to BMEC ratio of 10:1. The co-culture was incubated at 37°C for 3 h prior to isolation of RNA from the BMEC for qPCR.

Results: Culture of BMEC with *S. uberis* increased gene expression of IL-1, IL-6, IL8, 15LOX1, COX2, when compared to an untreated BMEC control. Adhesion molecules ICAM-1 and VCAM-1 also were upregulated upon bacterial exposure.

Conclusions: A thorough understanding of the pathological changes caused by uncontrolled inflammatory responses during intramammary *S. uberis* infections could lead to novel pharmacological targets that may reduce tissue damage and minimize production losses in dairy cattle.

004

MAP suppression of caspase activity in infected primary bovine macrophages

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Johne's disease is a chronic inflammatory infection in ruminants caused by infection with *Mycobacterium avium* subspecies paratuberculosis (MAP). Recently, our group presented evidence supporting MAP-infection mediated suppression of host cell apoptosis. However, little is known about the molecular mechanism behind this suppression of host apoptosis. In order to begin understanding mechanisms employed by MAP to circumvent host cell apoptosis, we hypothesized that MAP-infection would lead to a block in caspase activation. Because infected and uninfected cells in the same culture may influence each other, it was important to conduct studies using techniques that allow examination of single cells. Thus, bovine monocyte derived macrophages were infected with fluorescently labeled MAP and caspase signaling was measured using flow cytometry and CaspaTag™ staining. We observed a significant reduction in caspase activity in MAP infected macrophages compared to uninfected macrophages in the same culture (bystander cells) and to macrophages from control uninfected cultures. Bystander macrophages also displayed reduced caspase activity when compared to uninfected control cultures. To further examine the method of caspase activity reduction, we have also conducted apoptosis induction studies using agents such as TNFα and H2O2. Our results suggest that MAP-infected macrophages are resistant to caspase activation via different routes of apoptosis.

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induction. We conclude that MAP-infection significantly impairs the ability of macrophages to respond to apoptotic stimuli via caspase signaling. Studies probing changes in members of the apoptosis cascade as a result of MAP infection will help us further define the changes observed in MAP infected macrophages aiding in establishing a more mechanistic understanding of apoptosis control in host cells during MAP infection.

005

Brucellosis in sheep & goats of Bogra and Mymensingh Districts of Bangladesh.

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Purpose: Brucellosis is a zoonotic disease caused by *Brucella* that are pathogenic for a wide variety of animals and human beings. In Bangladesh, approximately 80% of people live in villages, 6.5% of national income and 3.5% gross domestic product (GDP) come from livestock. The people are in close contact with livestock on a daily basis and there are 33.5 million goats, 1.1 million sheep. After tuberculosis, brucellosis is the most important bacterial disease of livestock in Bangladesh but scant information is available about brucellosis in sheep and goat.

Methods: The seroprevalence of brucellosis in goat and sheep in Mymensingh and Bogra districts of Bangladesh were determined using slow agglutination test and Rose Bengal test as screening test and iELISA as confirmatory test. A total of 200 sera samples were collected from sheep and goat and the questionnaire based data on age, gender, area, reproductive problems such as abnormal uterine discharge, abortion or previous abortion were recorded.

Results: The prevalence of brucellosis in goat from Bogra district was 10% by RBT and 5.00% by SAT and Mymensingh district the prevalence was 2.85% by RBT, 2.85% by SAT, and 0.00% by iELISA. In case of 35 sheep, there was no reactor in male but out of 25 female the prevalence was 0.00% in RBT, 4.00% in SAT and 0% in iELISA respectively. Among 120 goats, 3 goats were aborted or previously abortion record and 117 goats were no abortion record. The prevalence of brucellosis was in aborted goats 100% in RBT and 100% in among SAT, iELISA respectively. While the non-aborted goats showed the prevalence 3.41% in RBT, 1.70% in SAT, and no positive reactors in iELISA. 1 case of abortion in sheep was found with prevalence of 100% in RBT, 100% in SAT and 100% in iELISA where as the 79 non-aborted sheep showed the prevalence of brucellosis were 2.53% in RBT, 1.26% in SAT. In this study, there were a significant ($p < 0.01$) association among abortion and the prevalence of brucellosis when the sera samples tested by RBT, SAT.

Conclusions: Goat and sheep may consider as a carrier of brucellosis and indicate as a risk for human and this is the first time that we used ELISA for diagnosis of brucellosis in sheep and goat from Bangladesh.

006

Identification of enhanced pathogenicity of *Brachyspira* species associated with clinical disease in swine.

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Purpose: Two species of *Brachyspira* are known to produce diarrheal disease in growing pigs. *Brachyspira hyodysenteriae* causes swine dysentery (SD), a severe mucohemorrhagic diarrhea with high morbidity and variable mortality; whereas, *B. pilosicoli* causes porcine intestinal spirochetosis (PIS) that is a less severe mucoid, cement-gray diarrhea with depressed growth and no mortality. Culture characteristics of *B. hyodysenteriae* are strong beta-hemolysis with positive ring-phenomenon while *B. pilosicoli* is weakly hemolytic with negative ring-phenomenon. PCR on isolates provides a more definitive confirmation of species. While the incidence of SD and PIS appeared to decline in the late 1990's, possibly due to the changes in swine production and the transition to high-health operations, submissions with a history of clinical disease consistent with SD or PIS for which *Brachyspira* culture is requested have been increasing recently. Additionally, a subset of *Brachyspira* isolates has emerged from pigs with clinical and histopathologic signs of SD or PIS that have inconsistent culture characteristics and are not confirmed as either *B. hyodysenteriae* or *pilosicoli* by PCR. The objective of this study was to further characterize *Brachyspira* isolates associated with clinical disease in swine.

Methods: Seventy-nine *Brachyspira* isolates identified from swine clinical samples submitted to the Iowa State University Veterinary Diagnostic Laboratory (ISU VDL) were cloned for purity and selected for further molecular diagnostics.

Results: Results of single-plex and multiplex PCR assays along with 16S rRNA sequencing suggest these isolates may be species previously described as non-pathogenic, including *B. murdochii* and *B. intermedia*. Additionally, several isolates could not be classified into the known *Brachyspira* species *B. hyodysenteriae*, *B. pilosicoli*, *B. intermedia*, *B. murdochii*, or *B. innocens*.

Conclusions: Changes in phenotypic characteristics and pathogenic potential have been identified in *Brachyspira* isolates associated with clinical disease in swine. Further investigations are warranted to evaluate these altered phenotypic and genotypic characteristics.

007

Functional analysis of methyl accepting chemotaxis proteins in *Campylobacter jejuni*.

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Purpose: *Campylobacter jejuni*, a major cause of bacterial foodborne gastroenteritis in humans, responds to environmental stimuli via chemotaxis, a mechanism which bacteria use to move towards a favorable environment. Motility and chemotaxis have been implicated in host colonization, invasion and other virulence-associated functions in many pathogenic bacteria. *In silico* analysis of *C. jejuni* 81-176 genome revealed several putative chemosensory receptors. However, the contribution of these chemosensory receptors to *C. jejuni* colonization and virulence/pathogenesis is not fully understood. The objective of the present study was to explore the contribution of methyl accepting chemotaxis proteins to *C. jejuni* pathogenesis/virulence.

Methods: We generated deletion mutants of nine methyl accepting chemotaxis proteins (MCPs) and assessed the contribution of these proteins to *C. jejuni* virulence/pathogenesis-associated functions.

Results: Six out of nine mutants showed defect in motility, while 3/9 mutants exhibited hyper-motility compared to the parental strain. In addition, chemotaxis assay using modified syringe method revealed that the mutant strains were defective in chemotaxis towards several chemicals, although 3 of the mutants showed hyper-chemotaxis towards some chemicals. Furthermore, invasion and intracellular survival assay using INT407 cells indicated that 3 of the mutants showed defect in invasion and intracellular survival compared to the parental strain.

Conclusion: To conclude, our findings suggest that MCPs play an important role in *C. jejuni* pathobiology.

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008

The ferric uptake regulator (fur) and iron uptake of *Edwardsiella ictaluri*.

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Edwardsiella ictaluri is the main bacterial pathogen of the catfish industry. Identification of virulence factors of *E. ictaluri* is vitally important to understand the pathogenesis and to the development of methods for controlling the disease. The ferric uptake regulator (Fur) is a metal ion-responsive transcription regulator that controls the expression of genes involved in diverse cellular functions, including genes related with iron uptake. Fur is synthesized as an inactive apoprotein and becomes activated when bound to iron. In absence of iron Fur becomes inactive, as a result several genes that was repressed by Fur are up regulated, especially genes related with iron uptake. Here we investigated the Fur protein and the iron acquisition system in *E. ictaluri*. Additionally, we evaluate the use of fur gene as a measure to develop live attenuated *E. ictaluri* vaccines for the catfish industry. Initially, we observed that *E. ictaluri* grown in iron-restricted conditions up regulate an outer membrane protein (OMP) of ~45 kDa. Also, the same protein was up regulated in *E. ictaluri* Δ fur mutants grown in presence of iron. This indicates that the 45 kDa OMP is regulated by Fur and probably important for *E. ictaluri* iron uptake. The amino acid sequence analysis showed that the *E. ictaluri* 45 kDa OMP corresponds to a hemin uptake protein TomB-dependent (HmuR). In silico analysis showed that hmuR gene is part of the operon hmuRSTUV, which possessed a putative Fur binding box in its promoter region. Whole cells grown under iron-restricted conditions and Δ fur mutants regardless of the iron condition in the growth media were able to bind hemin, indicating that HmuR is required for hemin uptake. This was confirmed by the observation that OMP isolated from *E. ictaluri* wild type grown only under iron-restricted conditions or Δ fur mutants were able to bind hemin as well. In vivo assays showed that *E. ictaluri* Δ fur presented increase of the LD50 in one fold-log in zebrafish (*Danio rerio*) and two fold-log in catfish (*Ictalurus punctatus*). Catfish immunized intra-peritoneal or by immersion was evaluated against the wild-type challenge. Here we described the Fur protein and its role in iron uptake and pathogenesis.

009

Identification of a novel *Campylobacter* enterobactin esterase cee by whole genome sequencing and comparative genomic analysis

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Ferric enterobactin (FeEnt) high-affinity iron acquisition system plays a critical role in *Campylobacter* pathogenesis. Recently, we identified a new FeEnt receptor CfrB that is involved in both iron acquisition and *Campylobacter* colonization in the intestine. One unique *C. jejuni* strain (JL11) was identified, which can efficiently utilize FeEnt by producing a functional CfrB only although it does not have another FeEnt receptor CfrA. Interestingly, *C. jejuni* 81-176 with published whole genome available was unable to utilize FeEnt although it also produces CfrB only. We hypothesize that JL11 contains an additional unidentified component that is missing in *C. jejuni* 81-176, but essential for CfrB-dependent FeEnt acquisition. In this study, the genomic DNA from JL11 was sequenced by current 454 GS FLX sequencer with Titanium series reagents. The 454 assembly software Newbler yielded unordered contigs, which consisted of 38 large contigs (> 500 bp) and 8 small contigs (< 500 bp). All gaps were closed between contigs and the draft genome sequence was subjected to automatic annotation. Comparative analysis of *C. jejuni* JL11, 81-176 and NCTC 11168 genomes identified that 81-176 lacks 10 genes that are present in JL11 and 11168. Based on published microarray data, of these genes only *cj1376* is potentially induced under iron-limited conditions. Complementation of 81-176 with *cj1376* successfully restored its ability to utilize FeEnt. Sequence analysis and structural prediction strongly suggested that Cj1376 is a novel *Campylobacter* Ent esterase (named 'Cee') located in the periplasm, which was further demonstrated by enzymatic analysis and amino acid substitution mutagenesis. PCR analysis showed that Cee is prevalent in *Campylobacter*. Genetic manipulation in different *C. jejuni* strains revealed that Cj1376 was essential for CfrB-dependent FeEnt acquisition and also involved in CfrA-dependent FeEnt acquisition. Taken together, whole genome sequencing and comparative genomic analysis successfully identified a novel Ent esterase Cee in *Campylobacter*, and provided insights into the molecular interaction of FeEnt acquisition systems in *Campylobacter*.

010

Identification of a high affinity adhesin of *Fusobacterium necrophorum* subsp. *necrophorum* that mediates binding to Bovine Endothelial Cells.

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Purpose: Outer membrane proteins (OMP) of many Gram-negative bacteria play an important role in their attachment to eukaryotic host cells which is considered as the first step in the establishment of infection. *Fusobacterium necrophorum*, a gram negative and rod-shaped anaerobe, is an important bovine pathogen that causes rumenitis-liver abscess complex, footrot, and necrotic laryngitis. Two subspecies are recognized, subsp. *necrophorum* and subsp. *funduliforme*, of which subsp. *necrophorum* is more often involved in fusobacterial infections. The purpose of this study was to evaluate the potential role of *F. necrophorum* OMPs in bacterial attachment to bovine endothelial cells.

Objective: The specific objective of this study was to identify OMPs of *F. necrophorum* that play an important role in bacterial attachment to the bovine adrenal capillary endothelial cells (EJG; ATCC-CRL 8659).

Methods: The outer membrane proteins were incubated with bovine endothelial cells and washed with phosphate buffered saline and detergents with increasing stringency to remove unbound OMPs. The tightly bound OMP was eluted using a sample buffer. The sequence of the identified protein was analyzed with Protein sequence analyzer.

Results: We identified a 40 kDa OMP from subsp. *necrophorum*, which bound with high affinity to endothelial cell membranes. N-terminal sequencing of this protein revealed similarity to OMPs of other *Fusobacterium* species. SDS-PAGE analysis showed absence of this protein in subsp. *funduliforme*. However, PCR assay indicated the presence of the gene in subsp. *funduliforme*. Far-western blot analysis revealed that the 40 kDa protein binds to bovine fibronectin.

Conclusion: We have identified a 40 kDa proteins which binds with bovine endothelial cells with high affinity which in turn may play a role in the binding of *F. necrophorum* to the host cells during the disease pathogenesis and efforts are on to further characterize this protein.

011

Interplay of *M. tuberculosis* and macrophages in the human respiratory system.

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Tuberculosis continues to be a devastating disease to humans worldwide. Inhaled *Mycobacterium tuberculosis* (*M.tb*) is phagocytosed by alveolar macrophages (AMs) via specific phagocytic and pattern recognition receptors (PRRs). Resident AMs are prototypic alternatively activated cells with

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increased expression of a subset of PRRs such as the mannose receptor (MR) that enhance microbial clearance and have a regulated inflammatory program with reduced pro-inflammatory mediated microbial killing. This biological state allows AMs to effectively clear microbes and particles within the alveolus while minimizing collateral inflammatory damage, but may also be exploited by the host-adapted *M.tb*.

Ongoing studies in the laboratory are exploring the various effects of the lung surfactant collectins SP-A and SP-D in shaping AM phenotype and function which impact the phagocytosis and intracellular fate of *M.tb*. For example, our studies are providing evidence that the preferential use of the macrophage MR pathway (following engagement by *M.tb*-exposed mannosylated lipoglycans) that occurs in the alveolar microenvironment during the infection process, directs intracellular trafficking of *M.tb* and its ultimate fate in the cell. *M.tb* clinical isolates vary in their degree of surface mannosylation impacting the use of these pathways and potentially disease outcome. Recent work in the laboratory is focusing on key transcriptional regulators of inflammation that are particularly relevant to AMs. In this regard, Peroxisome Proliferator-Activated Receptor gamma (PPAR γ) is a lipid-activated transcription factor that plays a key role in blocking pro-inflammatory responses and has high expression in alternatively activated AMs and macrophage-derived foam cells. We have determined that virulent *M.tb* and its cell wall ManLAM induce PPAR γ expression in macrophages through a MR-dependent pathway that regulates *M.tb* growth in these cells. SP-A also increases PPAR γ expression. Thus, bacterial surface mannosylation and surfactant components that “shape” AM biology alter the battlefield in favor of *M.tb* providing examples of *M.tb* adaptation to its environment.

012

Reinfection of mice with *Listeria monocytogenes* during pregnancy is associated with inflammation in murine fetoplacental units despite a decreased microbial load.

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Purpose: Foodborne outbreaks of *Listeria monocytogenes*, particularly with serotype 4b, continue to occur. Pregnant women are over represented in listeriosis outbreaks (17-fold increase in incidence of disease). Infection of the fetus and placenta results in abortion, stillbirth, or premature parturition. The latter carries with it a high risk for neonatal sepsis and meningitis.

Methods: We have developed a mouse model for infection with a serotype 4b strain of *L. monocytogenes* isolated from a foodborne listeriosis outbreak that resulted in abortion and fetal death. We used our infection model to compare maternal and fetal health in primary and secondary infections during pregnancy.

Results: Primary intragastric infection of pregnant mice resulted in severe infection of the maternal and fetal tissues in C57BL/6 mice. *L. monocytogenes* infection in a group of pregnant mice that have been infected prior to being bred have significantly reduced microbial load in maternal and fetal tissues. However, significant inflammation still occurs in the fetoplacental units.

Conclusions: These data suggest that their immune response to listeriosis is effective at decreasing microbial invasion of fetal tissues, but still incites an inflammatory response that is potentially detrimental to the fetus.

013

Identification of genes required for *Campylobacter* resistance to fowlicidin-1, a chicken host defense peptide.

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Purpose: Antimicrobial peptides (AMPs) are critical components of host defense limiting bacterial infections at the gastrointestinal mucosal surface. Bacterial pathogens have co-evolved with host innate immunity and developed means to counteract the effect of endogenous AMPs. However, AMP resistance mechanisms are still unknown in *C. jejuni*, an important human foodborne pathogen with poultry as a major reservoir.

Methods: In this study, random transposon mutagenesis and targeted site-directed mutagenesis approaches were used to identify genes contributing *Campylobacter* resistance to fowlicidin-1, a representative AMP in chickens.

Results: An efficient transposon mutagenesis approach (EZ::TNTM <KAN-2> Transposome) in conjunction with a microtiter plate screening identified three mutants whose susceptibilities to fowlicidin-1 were significantly increased. Backcrossing of the transposon mutations into parent strain confirmed that the AMP-sensitive phenotype in each mutant was linked to the specific transposon insertion. Direct sequencing showed that these mutants have transposon inserted in the genes encoding CbrR two-component regulator, CjaB transporter, and a putative trigger factor. Based on the analysis of 12 *Campylobacter* genomes, a conserved gene *cj1583c* displayed high homology (up to 46% similarity at amino acid level) to *sap*, an AMP resistance gene identified in other pathogens. Insertional inactivation of *Cj1583c* significantly increased susceptibility of *Campylobacter* to fowlicidin-1 in diverse strain background.

Conclusions: Together, these results define four *C. jejuni* genetic loci that will be useful for characterizing molecular basis of *Campylobacter* resistance to AMPs, a significant knowledge gap in *Campylobacter* pathogenesis.

014

Causes of failure on breeding soundness examination in Western U.S. Rams (2000-2007).

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Purpose: Breeding soundness evaluation of rams allows the veterinarian to assess the capacity of a ram to impregnate a defined number of ewes through natural service. The influence of ram fertility on pregnancy rate, lamb crop, and certain ranch economic indices is well established. The purpose of this retrospective study was to determine the reasons for unsatisfactory or questionable Society for Theriogenology classification in breeding soundness examination for 11,829 rams from over 100 flocks in CO, WY, and UT that were examined from 2000-2007.

Methods: Breeding soundness examinations consisted of a physical examination, body condition score, palpation of the scrotal contents, scrotal circumference measurement, manual exteriorization of the penis, collection of semen by electroejaculation, microscopic evaluation of sperm motility and morphology, and for selected rams, serologic testing for infection with *Brucella ovis* by ELISA.

Results: Approximately 1 in 4 rams tested were classified as unsatisfactory or questionable potential breeders. Rams from larger flocks were more likely to have failed from potentially infectious or inflammatory causes (epididymitis, leukospermia, or seropositive for *B. ovis*) than were rams from smaller flocks. Of all rams seropositive for *B. ovis*, a small proportion had palpable abnormalities of the epididymes or testes and a larger proportion had leukospermia; however, nearly half of seropositive rams were otherwise normal on breeding soundness examination.

Conclusions: *Brucella ovis* infection in rams may induce no detectable pathologic changes on physical examination or examination of the semen. Rams from larger range flocks may be at greater risk for infectious or inflammatory causes of infertility than rams from smaller flocks.

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015

Characterization of β -lactam resistance determinants in *Escherichia Coli* and *Klebsiella* Spp. from bovine mastitis in Canada.

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Purpose: Mastitis or 'inflammation of the mammary gland' is considered to be the most costly disease in dairy cattle. Antimicrobial agents represent the major approach for treatment and prevention of bovine mastitis and antimicrobial resistance (AMR) can lead to therapy failure. Identifying the genes causing resistance helps to understand the spread of AMR. Currently, little information is available on AMR determinants in *E. coli* and *Klebsiella* spp. from bovine mastitis in Canada.

The primary objective of this project is to characterize the antimicrobial resistance genes present in a subset of *E. coli*, and *Klebsiella* spp. bovine mastitis isolates collected by the Canadian Bovine Mastitis Research Network, with emphasis on the β -lactams.

Methods: The minimal inhibitory concentrations for β -lactams were determined for *E. coli* and *Klebsiella* spp. bovine mastitis isolates. 45 *E. coli* were resistant to β -lactam antimicrobials and 11 *Klebsiella* spp. isolates were resistant to cefoxitin. The AMR-ve Identibac microarray was used to characterize resistant *E. coli* isolates and a classical microarray was used to identify the resistance genes in 9 cephalosporin-resistant *Klebsiella* spp. isolates and 10 control isolates.

Results: Four different β -lactamase genes (26 *bla*_{TEM-1}, 4 *bla*_{OXA-1}, 3 *bla*_{CMY-2}, and one *bla*_{PSE-1}) were detected in *E. coli*. DNA sequencing showed that *bla*_{PSE-1} was part of a plasmid-borne integron carrying *aadA2*, *dfr16*, and *ereA*. Twelve isolates did not carry any of the β -lactamase genes under investigation but their susceptibility profiles were suggestive of an AmpC β -lactamase. DNA sequencing demonstrated the presence of a known mutation in the promoter region of the chromosomal *ampC* of *E. coli* leading to over-expression of this gene in these isolates. Of the 19 *Klebsiella* spp. isolates tested, four different β -lactamase genes (19 *bla*_{SHV}, 6 *bla*_{OXA-2b}, 2 *bla*_{FOX}, and one *bla*_{OXY}) were detected.

Conclusions: Overall, the prevalence of resistance to β -lactams was low in *E. coli* and high in *Klebsiella* spp.; however characterization of resistant *E. coli* and *Klebsiella* spp. isolates demonstrated a surprisingly high diversity of β -lactamase genes in both species.

016

Class 1 integrons and integrase gene in *Escherichia coli* isolated from Canadian beef cattle.

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Integrons are genetic elements involved in the mobility and clustering of antimicrobial resistance (AMR) genes. Class 1 integrons are often associated with mobile Tn21 transposons and are characterized by the presence of conserved regions with an *intI1* and *sul1* gene on either side of a variable region containing AMR gene cassettes. Previous investigations have identified a 'lonely *intI1*' gene, in *E. coli* isolates from beef cattle in western Canada, which lacked the *sul1* gene and gene cassettes.

The objectives of this work are: 1) assess the distribution and diversity of gene cassettes in class 1 integrons found in generic fecal *E. coli* isolated from feedlot beef cattle in western Canada, and 2) characterize the genetic environment of the 'lonely *intI1*' and assess its location and linkage to other AMR genes.

Based on sulfonamide susceptibility testing, 1000 *E. coli* isolates were chosen from four feedlots in Alberta, Canada. These isolates were tested by PCR for presence of *intI1* and positives were further screened for the presence of the *sul1* gene and gene cassettes. All the gene cassettes were identified by DNA sequencing. Ten isolates with a 'lonely *intI1*' were analyzed further by Southern blots, PCR, and sequencing.

A total of 203 *E. coli* possessed the *intI1* gene. Of those, 84 possessed a class 1 integron; the remaining 119 carried the 'lonely integrase'. The following gene cassettes combinations were observed in 78 of the integrons: *aadA1*, *aadA7*, *aadA12*, *aadB:aadA2*, *dfrA1:aadA1*, *dfrA1:aadA5*, *dfrA12:orf:aadA2*, *bla_{OXA-2}:orf* and *dfrA17:aadA5*. Overall, 18 different DNA sequences were observed in the variable regions of these 78 integrons. The 'lonely *intI1*' was found on the chromosome and two major types of plasmids which also carried the *tnpA*, *tnpR* and *tet(A)* genes of Tn1721.

A large diversity of gene cassette variants were detected in *E. coli* from the four feedlots investigated, which could be used to trace and model integron dynamics in bacterial populations from beef cattle. The 'lonely *intI1*' is likely to be associated with a highly mobile Tn21 derivative linked to additional chloramphenicol and sulfonamide resistance determinants. Further characterization of this genetic element is ongoing.

017

Staphylococcus aureus enhanced intracellular survival through coagulation manipulation.

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Staphylococcus aureus is a major cause of bovine chronic and subclinical mastitis, including abscess formation in the mammary gland. *S. aureus* hemolysins play a role in bacterial immune evasion and virulence. Our previous research showed *S. aureus* virulence locus, *agr*, and hemolysins, α and δ , modulate MAC-T immune responses during infection. *S. aureus* intracellular infections suppress certain MAC-T inflammatory and coagulatory gene responses. However, MAC-T thrombomodulin (TM) gene expression is induced by intracellular *S. aureus*. Supernatants collected from *S. aureus* intracellularly infected or extracellularly stimulated MAC-T were used to stimulate polymorphonuclear (PMN) leukocytes to quantify PMN gene expression. Interestingly, supernatants from intracellularly infected MAC-T induced a stronger inflammatory response in PMN than supernatants from extracellularly stimulated MAC-T. Therefore, we hypothesized that MAC-T TM activity during *S. aureus* infection is responsible for increased PMN activation. To test this hypothesis, we measured TM activity through generation of Activated Protein C (APC) on the surface of MAC-T. MAC-T were infected with *S. aureus* wild type strain, RN6390B (*agr*⁺), and knockout strains, RN6911 and RN4220 (both *agr*⁻), RN6390B -*hla* (hemolysin α -), -*hld* (hemolysin δ -), and -*hla/hld* (hemolysins α - and δ -) or stimulated with the respective supernatants. Bovine protein C and thrombin were incubated with MAC-T after 24h post infection. APC specific substrate-2366 induced a color change equivalent to APC concentration in the sample supernatant. Our results showed that intracellular infections with *S. aureus* RN6390B wild type, -*hla/hld*, and RN4220 strains generated significantly higher APC as compared to supernatant stimulated MAC-T. Intracellular infections with RN6911 and RN6390B -*hla* strains resulted in significantly less APC generation as compared to supernatant stimulated MAC-T. Increased TM may enhance *S. aureus* pathogenesis in vivo by increasing APC production which activates PMN, but may impair PMN. Future research will focus on the effect of APC on bovine neutrophil function.

BACTERIAL PATHOGENESIS

018

Microbiological and molecular characterization of coagulase positive *Staphylococcus* species isolated from canine clinical specimens.
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Multidrug-resistant *staphylococcus* species in dogs have become a serious challenge in veterinary medicine. The coagulase positive staphylococci most commonly associated with infections in the canine are the *Staphylococcus pseudintermedius* (SP), previously recognized as *S. intermedius* (both members of the *S. intermedius* group), *S. schleiferi* subspecies *coagulans* (SC) and *S. aureus* (SA). To better understand the magnitude of this problem, a total of 324 *Staphylococcus* sp from canine clinical specimens were analyzed based on the following objectives: 1) characterize canine *Staphylococcus* sp using conventional biochemical tests and molecular techniques, 2) identify epidemiological risk factors and 3) distinguish the most appropriated diagnostic method for recognition of methicillin-resistance (MR). Isolates were characterized by Polymerase Chain Reaction-Restriction Fragment Length Polymorphism (RFLP), latex agglutination for penicillin-binding protein 2a (PBP2a), agar disk diffusion for oxacillin (OX) and cefoxitin (FOX) antimicrobial susceptibility testing, and multiplex PCR for *ScmecA* typing and detection of Pantón-Valentin leucocidin (PVL) genes. Thirty-nine percent (125/324) of the isolates were MRSP, 9% (28/324) MRSC and 9% (30/324) MRSA. Fifty-one percent (151/297) of the isolates were collected from skin and 34 % (99/294) from dogs \geq 9 years old. PVL genes were found in 13% (4/30) of the MRSA, all possessing the type IV *ScmecA* cassette. The type V cassette was present in 66% (113/171) of the isolates, followed by type IV with 13% (22/171). Interestingly, 11% (18/171) of the MRSC were *ScmecA* type V. The sensitivity and specificity of OX for the detection of MR strains was 83% and 93%, respectively, but for FOX the sensitivity was 20% and the susceptibility 100%. To our knowledge this is the first time that *SCmecA* Type V has been reported in MRSC. Our findings confirm that cefoxitin is not appropriate for the detection of MR in canine staphylococci and recommend the use of PCR or the PBP2a latex agglutination test instead.

019

The complete genome of *Erysipelothrix rhusiopathiae*, a causative agent of swine erysipelas.

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Purpose: The facultative intracellular bacterium *Erysipelothrix rhusiopathiae* is a gram-positive, non-spore-forming and rod-shaped bacterium that represents the new class *Erysipelotrichia* in the phylum *Firmicutes*. The organism causes a variety of diseases in many animals including humans. Here we have determined the complete genome sequence of a virulent strain of *E. rhusiopathiae*, Fujisawa. Results:

The complete genome is a circular DNA molecule consisting of 1,787,941bp with a G+C content of 36.6% and encodes 1,704 predicted proteins and 7 rRNA operons. Phylogenetic analyses based on 16S rRNA gene sequences and concatenated alignments of 34 universal protein families, suggest that among bacteria in the *Firmicutes*, *E. rhusiopathiae* is positioned in the nearest group of the *Mollicutes*, which comprises mycoplasmas. Genome analyses revealed that the organism lost all the genes involved in biosynthesis of fatty acids and all but one of the genes for a functional tricarboxylic acid cycle. It also lacks genes for biosynthesis of many amino acids, several cofactors and vitamins, showing that the gene losses are attributed to the process of reductive evolution. Like many gram-positive pathogens, the genome is well equipped with various virulence features such as a capsule and cell-surface adhesins. However, a noteworthy feature of the genome is the presence of many antioxidant enzymes and phospholipases, suggesting that they may facilitate intracellular survival of the organism in phagocytic cells.

Conclusions: Thus, it appears that *E. rhusiopathiae* evolved in its own way toward eukaryotic parasitism and the genome structure may be the result of its adaptation to an intracellular life style.

020

High throughput transcript mapping of *Histophilus somni* 2336 by deep sequencing.

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To improve the annotation of *H. somni* 2336, Illumina sequencing of mRNA transcripts was conducted from triplicate cultures grown in Veterinary Fastidious Medium (VFM). A total of 9,015,318 Illumina reads were obtained with an average read length of approximately 76 bp. Reads were mapped onto the genome using Bowtie allowing 2 mismatches per read. Approximately 90% of the annotated genes had average read per base (RPB) values of 7 or higher. Genes were considered expressed if they had average RPB values of 7 or higher covering at least 60% of the gene length. Using this standard, a total of 1636 genes were identified as expressed. For operon prediction, two genes were considered part of a single operon if 1) both were expressed, 2) both were in the same orientation, and 3) the intergenic region between them was completely expressed. A total of 278 polycistronic operons were identified containing 730 genes. Intergenic regions were also scanned to identify novel expressed genes or small RNAs. More than 150 novel expressed regions with length >50 bp were identified. This project will improve our knowledge of *H. somni* operon structure and will result in the identification of novel sRNAs to accelerate pathogenesis research on this important bovine pathogen.

021

GenHtr_Quan a quantitative approach for comparative variant analysis of bacterial pathogens.

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Purpose: Here, I report a quantitative approach, referred to as GenHtr_Quan, for the comparative analysis of closely related bacterial strains to study the microevolution of bacterial pathogens via Solexa Sequencing, a next-generation sequencing technology.

Methods: GenHtr_Quan accomplishes this by establishing genome-wide heterogeneity genotypes for each genome, which are then quantified and comparatively analyzed. This allows the accurate identification of even minor changes in DNA sequences with great complexities.

Results: As a proof of the concept, I applied the procedure to the genomes of 15 *S. aureus* strains (simulated data) and four newly sequenced *S. aureus* strains (Solexa data), focusing extensively on the three pairs of hVISA/VISA strains. This analysis indicated that GenHtr_Quan could reveal mutations on the genes with great complexity including 16S and 23S ribosomal RNA genes.

Conclusions: It is expected that this tool will improve our understanding of the molecular mechanism underlying the dynamics and the evolution of drug-resistant bacterial pathogens.

BACTERIAL PATHOGENESIS

022

Profiling of porcine tonsil bacterial communities using Terminal Restriction Fragment Length Polymorphism (T-RFLP)

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Purpose: There is a growing awareness that microbial communities, rather than single microbes, should be considered as the “unit of pathogenicity”. Accordingly, studies were undertaken to begin characterize the microbiome of palatine tonsil in swine. This tissue, in addition to harboring a large number of poorly-characterized commensal organisms, is known to be the reservoir for many primary and opportunistic pathogens. Methods: As part of a larger study on the surveillance of respiratory infections in growing pigs, tonsillar samples were obtained from animals in “all-in, all-out” finisher barns. Swabs were evaluated by routine microbiological methods on polyethylene alcohol agar, MacConkey agar, and sheep blood agar with a *Staphylococcus aureus* streak. DNAs, prepared from pooled samples from these plates, were then analyzed by T-RFLP using Phusion® Bacterial Profiling kits. T-RFLP electropherograms were produced by capillary electrophoresis and the fragment peak sizes obtained were used for bacterial identification by searching the Finnzyme database, which contains data for > 60,000 organisms. Results: Pathogens such as *Streptococcus* spp., *Staphylococcus*, and *Actinobacillus* spp. were presumptively identified in virtually all samples; *H. parasuis* was detected in 10% of the DNAs tested. *Mycoplasma* and *S. dysgalactiae* peaks were also identified in a few samples. The other tonsillar residents presumptively identified in descending order of frequency of occurrence were *Bacillus* spp., *Actinobacterium*, bacteria found in swine effluents used for composting (e.g., *Leifsonia* and *Jonesia* spp.), bacteria involved in increased energy harvest and obesity, *Bacteroidetes*, *Aerococcus urinae*, *Firmicutes* (oral clone), *Actinomyces* (oral), and *Pseudomonas* spp. Members of the family *Enterobacteriaceae* were rarely detected, while *Campylobacter* spp., gut *Clostridium* spp. and *Lactococcus* were found in some samples. Conclusions: Although further refinements are required to increase the sensitivity and specificity of the T-RFLP method, it holds promise for obtaining a more complete picture of microbial communities than is currently available by routine bacterial culture methods.

BIOSAFETY AND BIOSECURITY

023

Effect of biosecurity, movement restrictions, and vaccination in the control of foot and mouth disease in livestock production systems in the central United States.

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Purpose: The potential impact of an introduction of Foot and Mouth Disease (FMD) in the central US and the effects of vaccination strategies were compared by use of simulation modeling. Simulation models were developed using the North American Animal Disease Spread Model (NAADSM), a spatially explicit, stochastic model.

Methods: Based on data from the 2002 U.S. National Agricultural Statistics Service and Kansas Confined Animal Feeding Permits, a simulated population of livestock operations for the central US were generated. The population included 493,166 herds defined by latitude and longitude, production type (Cow-calf, Large Feedlot, Small Feedlot, Dairy, Large Swine, Small Swine, Sheep, and Goats), and herd size. Simulated outbreaks began in a 35,000 head feedlot in Southwest Kansas.

Direct and indirect contact rates were estimated between each production type pair based on expert opinion. Herds detected as positive for FMD were quarantined preventing further direct transmission. Indirect contacts included movement of people, vehicles, and equipment between farms. Two levels of movement restriction of indirect contact were modeled by implementing movement controls following the first detection to decrease movement by 70%, or by 90% from pre-outbreak levels. Two levels of probability of disease transmission following indirect contact were also modeled (10% and 20%). In addition to a no vaccination baseline strategy, vaccination strategies varied according to the number of infected herds necessary to initiate a vaccination program (10, 50 or 100 herds) and the radius of the vaccination zone (10 or 50 km).

Results: When movement controls were decreased by 90% from baseline levels or probability of transmission was 10%, outbreaks were small and vaccination strategies were not effective in mitigating the outbreak. These results highlight the importance of biosecurity and movement restrictions.

Conclusions: Further data on the effectiveness and practical implementation of movement controls and biosecurity practices in the field setting is needed for optimal decision making.

024

Effect of vaccination strategy in the control of foot and mouth disease in livestock production systems in the central United States.

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Purpose: The potential impact of an introduction of Foot and Mouth Disease (FMD) in the central US and the effects of vaccination strategies were compared by use of simulation modeling. Simulation models were developed using the North American Animal Disease Spread Model (NAADSM), a spatially explicit, stochastic model developed for evaluation of control measures for highly infectious diseases.

Methods: Based on data from the 2002 U.S. National Agricultural Statistics Service and Kansas Confined Animal Feeding Permits, a simulated population of livestock operations for the central US was generated. The population included 493,166 herds defined by latitude and longitude, production type (Cow-calf, Large Feedlot, Small Feedlot, Dairy, Large Swine, Small Swine, Sheep, and Goats), and herd size. Simulated outbreaks began in a 35,000 head feedlot in Southwest Kansas.

Direct and indirect contact rates were estimated between each production type pair based on expert opinion. Herds detected as positive for FMD were quarantined preventing further direct transmission. Indirect contacts included veterinarians, feed trucks, milk trucks, salesmen, nutritionists, AI technicians, hoof trimmers, employee contact, and neighbors. In addition to a no vaccination baseline, vaccination strategies varied according to the number of infected herds to initiate vaccination (10, 50 or 100), the radius of the vaccination zone (10 or 50 km) and the target species for vaccination.

Results: Vaccination was most effective when it was initiated after 10 infected herds were detected and when implemented over a 50km radius. In those scenarios number of vaccine doses required was increased but the number of depopulated herds and duration of the outbreak were decreased.

Vaccination strategies targeted to only large feedlots (>3000 head) were as effective in decreasing depopulated herds and duration of the outbreak as those targeting all production types.

Conclusions: These results suggest that early vaccination over a wide area is most effective at controlling an FMD incursion. Further they suggest targeting vaccination to only large feedlots may be an effective and efficient way to implement vaccination.

025

Production region model of PRRSV and *Mycoplasma hyopneumoniae* transmission and biosecurity: Results of the 3-year study.

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Purpose: The objectives of this study were to evaluate airborne spread of PRRSV and *Mycoplasma hyopneumoniae* (M.hyo) under field conditions, and to test the efficacy of air filtration for the reduction of the risk of the airborne transmission and transport of those agents. Additionally, attempts to identify meteorological data associated with the airborne spread of both agents were made.

Methods: Swine Disease Eradication Center University of Minnesota production region model was used for the study. The production region model incorporated 4 different facilities to represent 4 different farms in endemically PRRSV-infected region. The infected population source facility was located in the middle of the region with 3 other facilities of different biosecurity levels; High-1 (MERV 16, 14, or electrostatic mechanical air filtration system, along with insect, fomite, personnel and transport protocols), High-2 (antimicrobial air filtration system), and Medium (matching protocols except for filtration), surrounding it at equal distance of 120 m. The study was planned to run for 3 years and 39 replicates each of 4 weeks in duration. PRRSV MN-184, 1-18-2, 1-26-2, and M.hyo 232 were used to inoculate the pigs in the infected population source. Serum and nasal swabs were collected weekly from all pigs in the 3 recipient facilities in order to monitor the infection status of PRRSV and M.hyo in each population. Air was collected using a cyclonic collector inside of each recipient facility as well as from the exhaust fan of the infected population source facility daily during the study period, and tested for those agents. Finally, on-site weather station was placed on the study site and real-time weather data were collected.

Results: The study will be completed by November 2010. The summarized results and conclusion will be presented during the meeting to support our observations as follows:

Conclusions: (1) airborne spread of PRRSV and M.hyo could occur under field conditions. (2) air filtration system successfully protected susceptible swine populations from the airborne spread of both agents. (3) certain weather patterns were documented during the episodes of airborne transmission of those agents.

BIOSAFETY AND BIOSECURITY

026

Prevention of PRRSV infection in large breeding swine herds located in swine dense regions by using air filtration: Preliminary observations
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Purpose: The objective of this study was to evaluate the efficacy of air filtration in large commercial sow herds in swine-dense regions and calculation of its cost: benefit using data from filtered and non-filtered herds.

Methods: Participant herds had to meet the following criteria. Filtered (treatment) herd: A PRRSV-negative sow herd with an inventory of ≥ 2400 sows to which a validated air filtration system has been installed. Filtered herds have historically received naïve gilt replacements and semen from naïve AI centers and have practiced a scientifically validated program of biosecurity for indirect routes of PRRSV transmission such as personnel/fomites, transports, and insects. Participant herds have experienced ≥ 3 new PRRSV introductions over the past 4 years. The herds were located in areas with ≥ 4 pig sites within a 2-mile radius and neighboring sites had experienced PRRSV infection and clinical disease 3-6 months prior to the initiation of the study. Unfiltered (control) herd: A sow herd which met the criteria defined for filtered herds, but which has not installed an air filtration system. To assess the impact of air filtration, we measured the followings: (1) differences in the frequency of virus introduction across treatment and control herd, defined as the detection of a PRRSV that differed by 2% in the ORF 5 region from previous viruses found in the herd. (2) cost of implementation of air filtration system on large sow herds. (3) differences in performance and profitability between treatment and control herds following analysis of production and financial data.

Results: The study was planned to run for 4 years (2008-2012), and is currently on going. As preliminary results from past 2 years, new PRRSV introduction has been documented in 2/10 filtered herds; however, transport and personnel breaches were confirmed via diagnostic data and security camera recording. On the other hand, new virus introduction was observed in 24/26 (92%) in non-filtered herds.

Conclusions: In preliminary conclusion, air filtration appears to be an effective means to reduce the risk of PRRSV-introduction to large commercial sow herds in swine-dense regions.

027

Evaluation of a needle-free injection system (AcuShot™) for reduction of hematogenous transmission of PRRS virus.

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Purpose: The purpose of this study was to evaluate the ability of a Needle-Free Injection Device (NFID), AcuShot™, to reduce or eliminate the hematogenous transmission of Porcine Reproductive and Respiratory Syndrome virus (PRRSv).

Methods: A total of 88, four-week-old, PRRSV-negative gilts were divided into four replicates of 5 groups. Group #1 (PRRSv source population) consisted of 10 pigs which were intramuscularly inoculated on day 0 with 2 ml of the MN-184 PRRSV isolate at a concentration of 10^4 TCID₅₀/ml. Group #2 (NFID), Group #3 (conventional needle/syringe or positive control), Group #4 (sham-inoculated), and Group #5 (negative control), each consisted of three pigs. All pigs in Group #4 (sham-inoculated) were intramuscularly inoculated with 2 ml of virus-free media. On days 5, 6 and 7 post-inoculation (PI) of Group #1, pigs from Groups #1, #2 and #3 were vaccinated with a *Mycoplasma hyopneumoniae* (*M. hyo*) bacterin. The same NFID and needle/syringe used to initially *M. hyo*-vaccinate the PRRSV source pigs (Group #1) on opposite sides of the neck were used to subsequently *M. hyo*-vaccinate pigs in Groups #2 and #3, respectively.

Results: At arrival (day 0), all pigs tested negative by PRRS ELISA and PCR. All Group #1 pigs tested PRRS PCR positive at days 2 and 5 PI. All 12 pigs injected with the conventional needle/syringe tested PRRS PCR-positive at days 12 and 19 PI, while 3 out of 12 pigs (1 replicate) injected by NFID tested PRRS PCR-positive by day 19 PI. The proportion of PRRS PCR-positive animals was significantly lower in the NFID group compared with the needle/syringe group ($P \leq 0.05$). On day 22 PI, the final day of the study, all PRRSV source pigs tested PRRS ELISA-positive, as did the 12 animals injected with conventional needle/syringe. Three of 12 animals treated using NFID and none of the negative controls or sham-inoculated pigs tested ELISA positive on day 22 PI.

Conclusions: The results of this study indicate that hematogenous transmission of PRRSV occurs from infected pigs to susceptible pigs via repeated use of the same needle and that needle-free systems reduce the hematogenous transmission of PRRSV.

029

Evaluating Porcine Circovirus Type 2 control in vaccinated herds: are sentinels needed?

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Purpose: The purpose of this study was to compare the detection of viral circulation of PCV2 in a farm after implementation of PCV2 vaccine through the use of sentinels as compared to testing of vaccinates.

Methods: Three replicates of 20 pigs each and one replicate of 22 pigs were weighed and ear tagged at weaning for use in the study. Pigs were matched in pairs by sex, weight and dam. Matched pairs of pigs were allocated to sentinel (non-vaccinated) and control (vaccinated) groups. Control pigs received a Killed Baculovirus vector PCV2 vaccine at weaning and 2 wks later. Blood samples were taken at weaning, 9 and 20 wks of age. ELISA, IFA-4 dilution and PCV2 PCR were performed. Pigs were weighed again at completion of the trial (20 wks of age) and ADG was calculated.

Results: ADG and mortality were not statistically different between sentinels and control pigs ($P > 0.05$; 713.5g vs. 695.4g, respectively). There was not a significant difference between both treatment groups in PCV2 antibody titers measured by ELISA and IFA-4 dilution ($P > 0.05$) at weaning. There was a significant difference in antibody titers measured by ELISA and IFA-4 dilution at 9 and 20 wk of age ($P < 0.05$). Two PCV2-PCR pools of sentinel and 5 control animals were positives ($P > 0.05$). Mortality rate was not significant different between both treatment groups ($P > 0.05$).

Conclusions: Monitoring sentinels did not result in an increased detection of virus circulation in this study, regardless of which diagnostic test was used. The use of sentinel swine requires increased labor and time for detection, as well as presenting the potential for impaired animal wellness issues if clinical illness occurs. This study suggests that the use of PCV2 PCR on vaccinates to determine virus presence or absence is equivalent to the use of sentinels.

BIOSAFETY AND BIOSECURITY

030

Methicillin resistant *Staphylococcus* spp in commercial pigs used in veterinary student training.

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Purpose: Research groups have recently identified methicillin resistant *Staphylococcus aureus* (MRSA) as a common colonizing agent in pigs, and that people with frequent contact have increased risks for colonization and infection. MRSA has also been identified as one of the most common causes of outbreaks of nosocomial infections in veterinary hospitals. The Colorado State University College of Veterinary Medicine and Biomedical Sciences utilizes commercial pigs in veterinary student training. These pigs are a potential source of environmental contamination as well as nosocomial and zoonotic infections. Objectives of this study were to estimate the frequency of MRSA colonization in young pigs used in teaching laboratories and evaluate associated environmental contamination.

Methods: Environmental samples were collected twice weekly from the holding facility and twice daily from the teaching laboratory using a commercially available electrostatic dust collection wipe (Swiffer, Procter and Gamble). Nasal and rectal swabs were collected twice from each pig (n=100). All samples were cultured for the presence of methicillin resistant *Staphylococcus* spp.

Results: Overall, the frequency of MRSA colonization in pigs at the holding facility and teaching laboratory was 49.5% and 71%, respectively. The pigs arrived in two groups (n=50) one month apart. The frequency of colonization was markedly different between groups. There was environmental contamination as well as evidence of residual contamination.

Conclusions: This study demonstrates a previously unrecognized risk associated with the use of young pigs in training and research in both veterinary and human medicine. Colonization of these pigs does not preclude their use rather it demonstrates the importance of biosecurity measures to control the associated risks.

COMPANION ANIMAL EPIDEMIOLOGY

031

Zoonotic disease awareness in animal shelter workers and volunteers and the effect of training.

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Purpose: Animal shelter workers are a vulnerable population whose exposure to zoonotic disease may be greater than the general population. The aim of this project was to identify baseline zoonotic disease knowledge of animal shelter workers and to develop and evaluate zoonotic disease awareness training.

Methods: Ten animal shelters in 6 western states were randomly selected. Trainees were evaluated by identical pre and post training tests. Training topics included: identification of high risk individuals to zoonotic disease, identification of clinical signs, susceptible species, and transmission of disease to animals and to humans. Zoonotic diseases included: rabies, plague, leptospirosis, internal parasites, methicillin resistant staphylococcus aureus and salmonella.

Results: A statistically significant difference in overall total scores between pre-test (58.5%) and post-test (69.5%) was observed ($p=0.001$). Overall test scores were raised 11%. No association was observed between test scores and length of time working in animal shelters, or with the participants' role at the animal shelter. Trainees correctly identified high profile immune compromised persons (children <5, adults >65, HIV/AIDS) at increased risk from zoonotic disease; fewer identified pregnant women, individuals with diabetes or heart disease at increased risk. The lowest baseline levels of knowledge were found with leptospirosis, MRSA, plague and rabies, emerging diseases with increasing prevalence and high consequence.

Conclusions: Zoonotic disease awareness training is a valuable service to animal shelters. In the current study, training was successful in transferring short term knowledge to animal shelter workers. To completely understand and evaluate the effectiveness of shelter worker training, long term assessment with observable and/or measureable behavioral outcomes is needed.

032

Comparison of antimicrobial resistance patterns of *Salmonella* spp. and generic *E. coli* recovered from pet dogs from volunteer households in Ontario, Canada (2005-2006).

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Purpose: The purpose of this study was to compare the antimicrobial resistance (AMR) patterns of *Salmonella* spp. and generic *E. coli* in the feces of pet dogs from volunteer households in Southwestern Ontario, Canada.

Methods: 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 generic *E. coli*. If available, up to three *Salmonella* spp. isolates per dog and three generic *E. coli* isolates per dog were sent for antimicrobial susceptibility testing.

Results: Generic *E. coli* was recovered from approximately 96% (133/138) of the dogs, and 23% (32/138) of the dogs had at least one fecal sample positive for *Salmonella*. In total, 515 bacterial isolates from 136 dogs from 83 households were sent for antimicrobial susceptibility testing.

Approximately 80% (414/515) of the *Salmonella* and *E. coli* isolates were pan-susceptible, and only pan-susceptible isolates were recovered from 72% (98/136) of dogs and 65% (54/83) of households. Based on a multilevel logistic regression model, we found that the percent of variation in resistance at the isolate, dog, and household levels were 16%, 31% and 53% respectively. In addition, the odds of resistance were 3.2 times greater in generic *E. coli* than *Salmonella*. Agreement in the presence of resistance between the bacterial species isolated in the same dog was relatively low (PABAK=0.38, 95% C.I. = 0.30-0.46).

Conclusions: Pet dogs are an important potential source of antimicrobial resistant *Salmonella* and generic *E. coli*. However, extrapolating the epidemiology of AMR in pathogens, like *Salmonella*, from generic *E. coli* should be done with caution at the individual animal or household level.

033

Seroprevalence of canine influenza virus (H3N8) in racing sled dogs.

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Purpose: Since first identified in Florida racing greyhounds in 2004, canine influenza virus (CIV) H3N8 has spread rapidly throughout U.S. dog populations. As CIV represents a recently emerged pathogen, the majority of dogs are susceptible, regardless of age, breed, or sex. While it is difficult to estimate the total number of CIV infections that have occurred since 2004, a recent one-year study conducted by our laboratory found CIV positive dogs in 11 of 16 Colorado humane shelters (69%) that were experiencing outbreaks of canine respiratory disease. One factor favoring infection is close-proximity of susceptible and infected dogs, as aerosolized respiratory droplets constitute the primary route of CIV transmission. Therefore, racing sled dogs living in community housing or working closely with one another are considered a potentially vulnerable population, especially as frequent travel, high levels of exercise, and environmental stresses may also contribute to CIV infection. The objective of this study was to evaluate the seroprevalence of and risk factors for CIV in sled dogs racing in the 2010 Iditarod.

Methods: Using a 2006 CIV isolate (A/Canine/Fort Collins/224986/06 [H3N8]), we performed hemagglutination inhibition (HI) assays on sera collected from over 400 dogs in the weeks just prior to the 2010 Iditarod start date. Additionally, to evaluate risk factors for CIV seropositivity, each musher completed a questionnaire on the location of the racing team's kennel and training, the team's medical history and CIV vaccination status, travel history, and social interactions with other dogs.

Results: Of the sera tested, including nearly 40 samples from dogs that were listed as receiving the CIV vaccine, none were seropositive for CIV H3N8 antibodies by HI assay.

Conclusions: From our results, it appears that CIV is not currently being transmitted among racing sled dogs, despite shared housing and frequent travel by the racing teams. Furthermore, additional studies are warranted to evaluate the efficacy of the recent CIV vaccine against CIV infection.

034

Practicality, feasibility, and validity of capture-recapture method to estimate dog population in Lumlukka District, Pathumthani Province, Thailand.

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Purpose: Estimating number of dog population was essential for designing effective rabies vaccination strategy. However, number of dogs in Thailand was difficult to estimate precisely, especially due to an existence of free-roaming dogs. Capture-recapture (CR) method was used to estimate dog population in the Philippines, Japan and Sri Lanka with some levels of successes. This study aimed to assess the practicality, feasibility, and validity of CR

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method for estimation of number of dog population in normal environment in Thailand. Official records showed that Lumluka district, Pathumtani provinces had the highest number of animal rabies cases, 33% and 4% of all cases in the province and in the country, respectively.

Methods: Hence, Lumluka district was selected as the studied area. All 126 villages in the studied district were stratified into rural and urban areas according to criteria set by Thailand's Ministry of Interior. Ten villages were selected using systematic random process within the strata. Captured dogs marked during vaccination campaign in the selected villages then recaptured 3 times on day 3, 7 and 10 post vaccination. Chapman method was used to estimate dog population. Practicality and feasibility of CR method was assessed against predefined criteria, including time usage, incurring costs, and contentment of field staff, using questionnaires. Two villages were selected for individual head counts of dogs comparing with results from CR method to ascertain validity.

Results: Results demonstrated that CR method was practical and feasible to be applied in Thailand. The validity depended on certain factors, but did not limit the usefulness of the method.

035

Prevalence of *Giardia* spp. and *Cryptosporidium* spp. in dogs in Chiang Mai, Thailand.

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Purpose: *Giardia* spp. and *Cryptosporidium* spp. are common causes of diarrhea in a wide range of hosts including human and animals. The prevalence of these organisms, however, in dogs in Chiang Mai, Thailand and the potential for dogs to serve as a reservoir host are unknown. Our objective is to explore the prevalence of *Giardia* and *Cryptosporidium* infection in dogs in this province. Methods: A cross-sectional study was designed and 301 canine fecal samples were obtained from owners during August 2009 to February 2010. Demographic and geographic data were recorded. The presence or absence of diarrhea was recorded at the time of sample submission. *Giardia* and *Cryptosporidium* infections were diagnosed using immunofluorescent assay (IFA). Factors associated with the disease were investigated. These factors are age of the dogs (<1 year, 1-7 years and >7 years), gender, diarrhea status, month of fecal obtaining and district of residence. Data were analyzed using Fisher's exact test and odds ratios were estimated. Results: The estimated prevalence of *Giardia* and *Cryptosporidium* infections were 8.6% and 7.6%, respectively. Conclusions: *Giardia* and *Cryptosporidium* infections in young dogs in Chiang Mai were common. These data suggested that dogs could be a potential reservoir for the zoonotic transmission of *Giardia* spp. and *Cryptosporidium* spp. The genotypes of the positive *Giardia* and *Cryptosporidium* samples are currently being determined.

036

Results of an international survey of Otterhound health.

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Purpose: The aim of this study was to gather health information on the world's population of Otterhounds to establish what health conditions are affecting the breed. The survey was undertaken in collaboration with The Otterhound Club in the UK and The Otterhound Club of America. Methods: This report relates to the main survey form, which owners were asked to complete for each live Otterhound that they currently own. The form was split into 5 sections: general information, breeding history, health testing and temperament, health problems and comments. Results: In total 612 survey packs were sent out and there were 359 responses, for an overall response rate of 59%. A total of 188 Otterhound owners in 14 countries provided information about their hounds, with forms being completed for 347 living Otterhounds. A total of 84 (24.2%) hounds were reported to have no health conditions. The median number of health conditions reported per hound was 1 (0 - 9) for the 259 (74.6%) Otterhounds with at least 1 reported health condition. The most frequently reported health conditions were sebaceous cysts (113 cases), ear infections (106 cases) and hip dysplasia (45 cases). Sebaceous cysts and ear infections are by far the most commonly reported health conditions in the present survey, and this is also the case in previous surveys. Even discounting ear infections and anal gland conditions (which can be considered to be skin conditions), skin conditions were 4 of the top 21 most prevalent conditions, together having a prevalence of 56.9%. The overall prevalence of many of the health conditions affecting Otterhounds in the present world survey were similar to that for all countries when the data were split according to country. Conclusions: Further analysis of the data collected in the present survey could include pedigree analysis to attempt to assess potential inheritance of some of the health conditions reported. Moving forward, it could be extremely valuable to follow the Otterhounds about whom we now have data on forward in their lives, and this is now underway.

037

Results of the 2004 KC/BSAVA purebred dog health survey: Caesarean section rates for UK pedigree dogs.

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Purpose: Data on the occurrence of caesarean sections in dogs are rare. Anecdotally, brachycephalic dogs are thought to be more likely to need a caesarean section as a result of mismatch between the bitch's pelvic size and the size of the puppies' heads. It is also thought that elective caesarean sections tend to happen before natural parturition begins in a significant proportion of pregnant brachycephalic bitches. The aim of this study was to describe the frequency of occurrence of caesarean sections in a large sample of pedigree dogs in the UK.

Methods: The authors classified breeds, using a skull classification system to compare ratio of the length of the facial skeleton to the cranial cavity, into 3 categories: mesocephalic, brachycephalic and dolicocephalic. Data on the numbers of litters born in the 10 years from 1995 to 2004 were available from a cross-sectional study of pedigree dogs in the UK. The section of the questionnaire on breeding history of all dogs owned and bred in the previous 10 years included the questions "How many litters have your female dogs had in total?" and "How many litters were delivered by Caesarean section?" which were used in this study. The frequency of occurrence of caesarean sections was estimated as the percentage of litters that were reported to be born by caesarean.

Results: A total of 151 breeds were included on which data were available for at least 10 litters (range 10-1415), representing 13141 bitches that whelped 22005 litters. There were 7 breeds that had a caesarean rate of 0% and there were 3 breeds with a caesarean rate >80% (first 3 breeds in list below). The 10 breeds with the highest caesarean rates (>40%, in descending order) were the Boston Terrier, Bulldog, French Bulldog, Mastiff, Scottish terrier, Miniature Bull Terrier, German wirehaired pointer, Clumber spaniel, Pekingese and Dandie Dinmont Terrier. Five of these breeds were brachycephalic

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breeds.

Conclusions: While the results of this study cannot necessarily be generalised to all dogs in the UK, because the 2004 health survey was a convenience sample rather than a random sample of the UK pedigree dog population, these data provide evidence for the need to monitor caesarean rates in certain breeds of dog.

038

Results of the 2004 UK KC/BSAVA purebred dog health survey: Mortality and morbidity due to gastric dilatation-volvulus syndrome.

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Purpose: Gastric dilatation (GD) and gastric dilatation-volvulus syndrome (GDV) are acute, life-threatening conditions that most commonly affect giant and large breed dogs. GD and GDV occur as a result of the rapid accumulation of air in the stomach, malposition of the stomach to a varying degree and a rise in intragastric pressure, and this frequently leads to the development of cardiogenic shock. The aim of this study was to estimate breed-specific prevalence of and risk of death due to GD/GDV in UK pedigree dogs.

Methods: Data were available from a cross-sectional study of pedigree dogs undertaken in 2004 in the UK on the reported cause of and age at death and the occurrence of and age at diagnosis of disease. A total of 15,881 dogs of 165 breeds had died in the previous 10 years; GDV was the cause of death in 65 breeds. There were 36,006 live dogs of 169 breeds of which 48 breeds had experienced 1 episodes of GDV. Prevalence ratios were used to estimate breed-specific GDV mortality and morbidity risks.

Results: Gastric dilatation-volvulus was the cause of death for 389 dogs, representing 2.5% (95% CI: 2.2-2.7) of all deaths reported and the median age at death was 7.92 years. There were 253 episodes in 238 live dogs. The median age at first diagnosis was five years. Breeds at greatest risk of GDV mortality were the bloodhound, Grand Bleu de Gascogne, German longhaired pointer and Neapolitan mastiff. Breeds at greatest risk of GDV morbidity were the Grand Bleu de Gascogne, bloodhound, otterhound, Irish setter and Weimaraner.

Conclusions: These results suggest that 16 breeds in the UK were at increased risk of morbidity and/or mortality due to GDV in 2004. All but 2 of these were large or giant breeds with the Basset Hound and Chow Chow being the exceptions.

039

Clustering of serologically diagnosed cases of Coccidioidomycosis among dogs in Texas.

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Purpose: To determine whether serologically diagnosed cases of Coccidioidomycosis among dogs in Texas clustered in space, time or space-and-time.

Methods: The study used canine sera submitted to Texas Veterinary Medical Diagnostic Laboratory (TVMDL) from clinically suspected dogs between July 1, 1999 and December 31, 2009 for serological testing against *Coccidioides sp.* using agar gel immune-diffusion (AGID) and/or complement fixation (CF) tests. Cases were determined from the number of dogs testing positive by AGID or CF. The study population was determined from the estimate of dog population in 246 sample submitting zip codes using the formula described in "U.S pet ownership and demographic source book" and consisted of 1,468,382 dogs. Spatial, spatio-temporal (Poisson model) and temporal scan statistics implemented in SaTScan were used to examine for clustering of seroreactivity using centroid of the zip code location of sera submitting hospital and access date of the laboratory. Both circular and elliptical scanning windows were used to detect clusters.

Results: A spatial cluster ($P=0.001$) in the western part of Texas and two spatio-temporal clusters ($P=0.001$, 0.024) of sero-reactive dogs were identified. The spatial cluster overlapped the endemic region previously identified using human data. Within the spatial cluster, sero-reactive cases were clustered in the period of 2004-2009. Purely spatial cluster in the southwest of Texas was more compelling ($RR=27$, circular; 30 elliptical cluster) than purely temporal ($RR=1.45$) or space-and-time cluster ($RR=12$, circular window; 15, elliptical window) suggesting geographic location is more important than temporal or space-time variations in describing the occurrence of the disease.

Conclusions: Findings from this study indicate the utility of geo-referenced laboratory data on canine Coccidioidomycosis for identification, characterization and delineation of boundaries for endemic areas using tools for cluster analysis. Furthermore, the findings suggest the potential use of dogs as sentinels for early warning and control of the disease in humans.

040

Evaluation of collars and microchips for visual and permanent identification of pet cats

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Purpose: A general dogma apparently exists among cat owners and veterinarians that cats cannot wear collars or will be injured by them. The objectives of the study were to determine the percentage of cats still wearing a collar 6 months after placement, to compare differences in the percentages among collar types, to document problems (if any) with cats wearing collars, to describe owner perceptions regarding their cats wearing collars, and to determine the percentage of cats with functional microchips 6 months after implantation.

Methods: A randomized clinical trial with 538 cats was conducted at 4 study sites. Cats were randomly assigned to wear 1 of 3 types of collars: plastic buckle, breakaway plastic buckle safety, and elastic stretch safety. Each cat was fitted with the assigned collar and a microchip was inserted SC between the scapulae. Owners completed questionnaires about their experiences and expectations of collars at enrollment and at the conclusion of the study.

Results: 391 of the 538 (72.7%) cats successfully wore their collars for the entire 6-month study period. Owners' initial expectations of the cats' tolerance of the collar and the number of times the collar was reapplied on the cats' necks were the most important factors predicting success. Type of collar likely influenced how often collars need to be reapplied. Eighteen (3.3%) cats caught their collar on an object, in their mouth or a forelimb. Of the 478 microchips that were scanned at the conclusion of the study, 477 (99.8%) were functional.

Conclusions: Most cats successfully wore their collars. Because even house cats can become lost, veterinarians should recommend that all cats wear ID collars since they are the most obvious means of identifying an owned pet. For some cats, collars may frequently come off and become lost; therefore, microchips are an important form of backup identification. Owners should select a collar that their cat will tolerate and should check it often to ensure a proper fit.

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041

Characterization of advertisements for puppies sold online: Determinants of cost and a comparison with parent club breeders

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Purpose: The Internet is an increasingly common way for consumers to purchase puppies. Yet very little information is available about the types of puppies sold over the Internet. In addition these sales are not subject to USDA regulation. The objectives of the study were to describe puppies sold over the Internet, to assess the characteristics that contribute to the cost of a puppy, and to compare puppies sold on the Internet with puppies sold by AKC Parent Club breeders.

Methods: Over 14 weeks in 2008, Yorkshire Terrier, Shih Tzu, English Bulldog, Boxer, and Labrador Retriever puppies for sale on two large-scale online puppy sales sites were categorized based on their Internet advertisements. Data were collected in three categories: puppy characteristics, health characteristics, and policies. After the survey was completed, 25 AKC Parent Club breeders and 25 breeders who advertised at one of the puppy sales websites were randomly selected and interviewed over the phone.

Results: Small breed puppies were most frequently advertised with 35.2% (1,228/3485) of advertisements for Yorkshire Terriers and 23.0% (802/3485) for Shih Tzus. Almost one quarter of Internet breeders 768/3,474 (22.2%) advertised four or more different dog breeds. Champion bloodlines increased the cost of a puppy of all breeds. AKC Parent Club breeders 21/25 (84%) were more likely to mention breed-specific health screening tests when compared to Internet breeders 7/25 (28%).

Conclusions: Consumers should apply the same standards to a breeder found through a puppy sales site as they would to a local breeder. Breeders who advertise at one of the large-scale puppy sales websites are less knowledgeable about breed-specific health issues compared to an AKC Parent Club breeder. Internet breeders are less likely to perform these screening tests on their breeding dogs and may breed dogs with undesirable heritable health risks.

042

Using number-needed-to-treat to quantify risk in the context of breed-specific legislation and public safety.

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Purpose: Dog bites are common in the United States and limiting their incidence is a great concern. One method to decrease the risk of dog bites is breed-specific legislation (BSL) which bans or restricts ownership of specific breeds of dogs presumed to pose the highest risk of inflicting serious bites. Despite the frequency with which this legislation is proposed, there are no data to support claims of efficacy of BSL. Other limitations include inability to reliably identify dogs of specific breeds, and lack of data on breed ownership.

Methods: Using the number-needed-to-treat from evidence based medicine, we defined the number-needed-to-ban (NNB) to show how many dogs of specific breeds would need to be banned and removed to prevent even a single bite. This novel approach used demonstrated methods of risk communication to inform public health efforts.

Results: Using data from an emergency department study (incidence of bites=130/100,000 people) and an estimate that a single breed represented a maximum of 15% of all dog bite victims entering an emergency department, the NNB to prevent one bite was at minimum 5,128 dogs. To prevent an insurance claim due to a dog bite related injury, > 59,000 dogs would need to be removed. More serious injuries, which are also less frequent, leads to increasingly large NNBs, even if up to 35% of bites were attributable to a single breed.

Conclusions: NNB provides an easily understood tool to put the limitations of BSL into context. Communication of scientific data about risk is crucial for effective public policy decisions.

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043

Risk factors associated with carriage of *Clostridium difficile* in horses admitted to a veterinary teaching hospital in Atlantic Canada.

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Clostridium difficile is an important hospital-associated pathogen that commonly causes antimicrobial-associated diarrhea in humans. Evidence also suggests that it may cause acute diarrhea in horses. Its presence may be seen in both horses with diarrhea as well as asymptomatic carriers. Carriage in horses, in particular, is an area that needs further investigation given the presence of *C. difficile* in the environment and the risk of exposure to humans and animals. The objective of this case-control study was to identify potential risk factors associated with carriage of *C. difficile* in asymptomatic horses presented to a Veterinary Teaching Hospital in Prince Edward Island, Canada.

Fecal samples collected from 588 horses admitted to the Veterinary Teaching Hospital during the period May 2007 to May 2009 were processed. Feces were incubated in *C. difficile* selective enrichment broth for seven to ten days, then alcohol-shocked, centrifuged, and incubated on *C. difficile* selective agar plates for another seven days. All morphologically typical colonies were confirmed as *C. difficile* by Gram stain and proline aminopeptidase activity. Finally, a real-time PCR (qPCR) assay for the *C. difficile* *tcdB* (toxin B) gene was performed on *C. difficile* isolates.

A preliminary analysis of 78 horses (39 cases/39 controls) paired by sample date was conducted. Hospital records were examined and signalment, historical factors, and hospital complications were collected. Results suggest that administration of flunixin meglumine ≤ 30 -days prior to admission ($P=0.088$, OR 3.39) and horses whose primary purpose was breeding ($P=0.018$, OR 4.73) present an increased risk for carriage of *C. difficile*. Findings also support a protective effect if a horse was administered phenylbutazone ≤ 30 -days prior to hospitalization ($P=0.046$, OR 0.14), is used for pleasure ($P=0.187$, OR 0.49), or had a medication history ≤ 30 days of sampling ($P=0.140$, OR 0.63). Lastly, data from an additional 68 horses will be integrated into this study and multivariable analysis of risk factors will be performed to complete this investigation.

044

An epidemiologic analysis of *Salmonella* spp shedding in hospitalized horses with or without diarrhea.

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Identification of risk factors associated with *Salmonella* shedding in horses with or without diarrhea can help improve current hospital surveillance programs for early detection of *Salmonella* spp in fecal specimens in hospitalized horses. The objective of this study was to investigate exposure factors (before admission, at admission, and during hospitalization) associated with *Salmonella* shedding in hospitalized horses with or without diarrhea. A preliminary 1-year study included (i) 15 horses that tested positive for *Salmonella* fecal shedding at admission or during hospitalization and had diarrhea; (ii) 17 horses that tested positive for *Salmonella* and did not have diarrhea; and (iii) 32 horses that tested negative for *Salmonella* and did not have diarrhea. The association between investigated exposure factors and horses with or without diarrhea was examined using logistic regression. In a preliminary analysis, among horses without diarrhea, the following factors were associated ($P \leq 0.10$) with *Salmonella* shedding: (i) prior to admission: history of anti-inflammatory use; (ii) at admission: leucopenia, total blood protein, white blood cell count, percentage of lymphocytes and monocytes; and (iii) during hospitalization: season. Among horses with diarrhea, the following factors were associated with shedding: (i) prior to admission: history of antimicrobial use, anti-inflammatory use, sedative use, and fever; (ii) at admission: leucopenia, white blood cell count, percentage of lymphocytes and monocytes; (iii) during hospitalization: season and fever. Age (foals) was associated with *Salmonella* shedding in horses with or without diarrhea. Final results of a 4-year study will be presented at the conference.

045

Purchase price, exercise history, lameness, speed and sales price in 2-year-old in-training Thoroughbred horses.

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Pinhooked yearling Thoroughbreds are purchased at public auctions from July to September for the purpose of selling them the following year at what are known as 2-year-olds in-training sales. Trainers have 5 months to break, condition, and prepare their horses to work at high speed during training in preparation for the sales. At the sales, the trained horses race 1/8 or 1/4 mile. After purchase, the horses start racing as 2-year olds. Knowledge of exposure factors associated with athletic performance and sales price in 2-year-old in-training horses is limited. The objective of this study was to examine the relationship between purchase price, exercise history, lameness, speed and sales price in 2-year-old in-training horses. Fifty-one horses were included in this study. Horses were monitored daily to measure distance galloped or breezed during training, as well as for diagnosis of lameness. Median sales price of study horses was \$37,000. Median sales price was higher (i) in horses with a high purchase price (\$145,000), compared to horses with a low purchase price (\$22,000) ($P < 0.05$); (ii) in horses with a low number of furlongs galloped 1 to 60 days before the sales (\$85,000), compared to horses with a high number of furlongs galloped (\$25,000) ($P < 0.05$); and (iii) in horses classified as fast (\$350,000), compared to non-fast horses (\$30,000) at the sales ($P < 0.05$). These results and the effects of purchase price and lameness on distance galloped during training in study horses will be presented at the conference.

046

Clustering of and Risk Factors for the Porcine High Fever Disease in a Region of Vietnam

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Purpose: Porcine high fever disease (PHFD) emerged in 2006 in China and spread to Vietnam. Little work has been done to investigate risk factors of PHFD and space-time dynamics. This study's objective was to fill this gap by investigating probable cases of PHFD at household level as the outcome. Methods: The area of interest was a district of a province of southern Vietnam that had reported the outbreak of PHFD in 2008. A study area, $\sim 10 \times 10$ km in dimension, containing 37 hamlets, was selected. A survey was conducted in the area to collect information about the swine health problems during 2008. A group of trained interviewers aimed to perform a census of all households having pigs with local veterinarians as guides. The questionnaire included 3 sections: general information, clinical signs of disease in pigs, and production factors hypothesized to be risk factors. Case definition was based on literature and included interpretation of clinical signs in series. Logistic regression with a random intercept at the hamlet level was used to assess risk factors for PHFD. Spatial clustering was investigated using D-function and the Cuzick-Edwards test. Spatial clusters were evaluated using spatial relative risk surfaces and spatial scan statistics using a Bernoulli model. Space-time clustering was explored using the space-time K-function and Knox's test. Space-time clusters were evaluated using a space-time permutation model in SaTScan.

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Results: Of 955 households with questionnaire data, 33.4% were classified as cases. No significant spatial or space-time clusters were detected. The statistical significance of space and space-time clustering differed between methods employed. The risk factors associated with the occurrence of cases were: higher number of sows and finishing pigs (log 2 transformed), receiving pigs from an external source, and the interaction between using "water green plants" (WGP) as pig feed and owning ducks with/without contact with pigs in the household.

Conclusions: The interaction between presence of ducks in the household and feeding WGP to pigs suggested involvement of pathogens that could be present in water (environment), and could further multiply in or on ducks.

047

Serological prevalence of Japanese encephalitis virus in pigs in four mountain districts in Nepal.

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Japanese Encephalitis Virus (JEV) is a mosquito-borne zoonotic arbovirus that can cause severe neurologic disease and is the leading cause of viral encephalitis in Asia. Approximately twenty-five percent of cases are fatal and 30% have long term neurologic effects. Recently human cases of JEV have been reported from mountain districts of Nepal which were previously thought not to harbor JEV due to the higher elevation creating unfavorable breeding habitats for mosquitoes. Further investigation, however, found no history of travel to endemic areas in the majority of these human cases.

Purpose: The objective of this study was to estimate the prevalence of anti-JEV antibodies in pigs, which are an amplifying host for JEV, in four mountain districts known to have human cases of JEV.

Methods: A cross-sectional survey was performed in July- August of 2010. A total of 454 pig serum samples were collected and tested by competitive ELISA for the presence of anti-JEV antibodies. A questionnaire was administered to pig owners to identify risk factors associated with seropositivity.

Results: Results showed that 16.7% (17 out of 102), 4% (4 out of 100), 6.6% (10 out of 151) and 44.6% (45 out of 101) of pigs in Sindhupalchowk, Dolakha, Solukhumbu and Kavrepalanchowk districts had anti-JEV antibodies. The combined prevalence in these four districts was 16.7% (95% CI: 13.2, 20.2). None of the 76 seropositive pigs came from known endemic districts where previous exposure may have occurred. Mosquitoes were observed by residents in all four districts.

Conclusions: The results suggest that JEV is likely circulating in the mountain districts of Nepal and infection with JEV should be considered a risk to residents and travelers in this area.

048

The association between submission counts to a veterinary diagnostic laboratory and the economic and disease challenges of the Ontario swine industry from 1998-2009.

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Purpose: An intuitive assumption is to believe that the number of submissions made to a veterinary diagnostic laboratory is dictated by the financial state of the industries using the laboratory. However, no research is available to document how the economics of a food animal industry affects laboratory submissions and therefore disease monitoring and surveillance efforts. The objectives of this study were to determine if swine submissions made to a veterinary diagnostic laboratory fluctuated over time and to subsequently determine if economic indices associated with the Ontario swine industry can account for the variability seen in these submissions.

Methods: Retrospective swine submissions made to the Animal Health Laboratory at the University of Guelph, Guelph, Ontario from Jan '98 to Jul '09 were compiled and averaged into monthly counts. The following economic, demographic, and health variables impacting Ontario swine production were selected for analysis: auction price, lean-hog futures, currency exchange rate, price of corn, disease outbreak, government incentive program, number of farms in province, and average farm size. All independent variables identified by unconditional associations to have a significance of $P \leq 0.2$ with the outcome of monthly submission count were put into a multivariable negative binomial model. A final model was identified by a backwards elimination process.

Results: A total of 30,432 swine submissions were made. The total number of observed months was 139 and the overall mean of the monthly counts was 212.94 (SD=55.95). After controlling for farm size and the number of farms in Ontario, higher submission counts were associated with a weaker CAD\$ vs. US\$, higher auction prices, and disease outbreaks ($P < 0.05$).

Conclusions: The results suggest that both economic volatility and disease outbreaks in the Ontario swine industry drive submissions to the laboratory. In conclusion, lab submissions are a useful source of animal health data for disease surveillance purposes keeping in mind however that surveillance activities should also monitor the economics of the industry in conjunction with disease trends and outbreaks.

049

Molecular epidemiology of porcine reproductive and respiratory syndrome virus (PRRSV) in Ontario, Canada.

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Purpose: To study molecular epidemiological aspects of porcine reproductive and respiratory syndrome virus (PRRSV) from Ontario, Canada.

Methods: Recent work on constructing a global type 2 PRRSV phylogenetic tree ($n > 8,500$) based on ORF5 sequence data using Bayesian phylogeny was used as a backbone to analyze Canadian type 2 ORF5 sequences ($n = 500$) from the Province of Ontario with computational methods in accordance with the original tree. Transmission dynamics between Canada and the United States were estimated using the ancestral reconstruction approach. Assessment of employing restriction fragment length polymorphism (RFLP) typing in gauging genetic diversity or relatedness was evaluated by the Mantel test.

Results: The diversity of these sequences from Ontario alone was comparable to the entire United States with organization into 5 out of the 9 lineages defined in the backbone tree. Most sequences were clustered within lineages 1 and 2 followed by occupancy into vaccine associated sub-lineages 5.1 and 8.9. The tree topology also suggested a Canadian origin of the MN184-like PRRSV outbreak as the MN184-like cluster was depicted buried within the Canadian diversity. Transmission dynamics revealed frequent border crossing viral transmissions, with a higher rate estimated from Canada to the US than in the reverse direction. Finally, the Mantel test indicated strong association between RFLP difference and genetic distance (p value = 0.01). However, caution is required in interpreting RFLP typing results as either distantly related strains sharing the same RFLP pattern or closely-related strains

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with different typing patterns were sometimes observed from the phylogenetic tree.

Conclusions: The study of molecular epidemiology through sequence data provided in depth detailed valuable information on the high Ontario PRRSV diversity, transmission flow with the United States, evolutionary origin of the MN184-line outbreak, and the use of RFLP in predicting genetic distance.

050

One year's study of dynamic and evolution of type I and II PRRSV in a seed-stock farm.

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One year's study of dynamic and evolution of type I and II PRRSV in a seed-stock farm. H.K. Kim, S.J. Park, S.M. Rho, J.Y. Han, V.G. Nguyen, B.K. Park. Department of Veterinary virology Lab, College of Veterinary Medicine and BK21 Program for Veterinary Science, Seoul National University, Seoul, 151-742, Korea

Purpose: Porcine reproductive and respiratory syndrome virus (PRRSV) is a swine pathogen which is hard to be controlled. This study was to investigate dynamic and evolution of PRRSV in a seed-stock farm by monitoring PRRSV status from 11st, Jun, 2009 to 4th, Aug, 2010. Methods: For laboratory test, around 18-24 umbilical cords from farrowed sows and 5-95 sera from nursery and grow/finish barns were submitted around every 2 weeks interval during the study. The submitted samples were tested for PRRSV using IDEXX PRRS 2XR ELISA kit, RT-nested PCR and molecular epidemiology. Results: During a half of this study (Jun to Dec, 2009), frequent type II PRRSV viremia and low serum PRRSV-specific IgG level (around 0.0 to 0.5 of mean S/P ratio) were observed in nursery barn, while little viremia but high serum IgG level (around 0.6 to 0.8 of mean S/P ratio) were in grow/finish barn. Interestingly, a sudden increase of serum IgG level (1.2 to 1.8) was first detected from Nov, 2009 in grow/finish barn, thereafter, type I PRRSV-positive sera, umbilical cords and elevated serum IgG level were started to be found in the nursery barn from Jan, 2010 to Jun, 2010. Thirty three ORF5 full sequences from 14 type II and 19 type I PRRSVs were obtained in this farm and the genetic characteristics and evolution rates of those sequences were analyzed. One of the finding was that the substitution rates (/site/day) of two types were 4.06×10^{-5} (type I), 3.08×10^{-5} (type II), respectively, which was more frequent than previous reports. The calculated divergence time of type I PRRSV was consistent with the time when the sudden elevation of serum IgG in grow/finish barn was first observed. Conclusions: This study provided fundamental data for PRRSV epidemiology in a seed-stock farm and suggested a grow/finisher barn as a primary site for PRRSV introduction.

051

Pen-based oral fluid sampling for PRRSV in low prevalence situations.

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Purpose: The objective of this study was to estimate the probability of detecting one PRRSV viremic pig in a pen of ~25 pigs using a single oral fluid sample from the pen.

Methods: This study was conducted in a confirmed PRRSV negative (serology and PCR) finishing site. All animal housing, handling, and veterinary care was dictated by the guidelines published in the *Guide for the Care and Use of Agricultural Animals in Agricultural Research and Teaching* (Federation of Animal Science Societies, Savoy, IL). Oral fluid samples were collected from 36 pens (~25 pigs per pen) in one barn for 3 consecutive days (Study Day 1, 2, 3). On Study Day 4, one pig from each pen (n = 36) was removed, placed in isolation in a separate building on the site, and vaccinated with a commercial modified-live PRRSV vaccine, as per manufacturer's recommendations. One oral fluid sample was collected from each pen (36 + the vaccine pen) for the next 4 days (Study Day 4, 5, 6, 7). On the evening of Study Day 7 (4th day post vaccination), one vaccinated pig was placed in each of the 36 pens of PRRSV-negative animals. The following morning (Study Day 8), one oral fluid sample was collected from each pen. During this 30 minute collection period, the vaccinated pig was observed for the number of interactions with the rope and the results were recorded as a dichotomous outcome (Y/N) for each one minute interval. Oral fluid samples (30 minute collection) were then collected daily from each of the 36 pens for Study Days 9 through 15. All samples were completely randomized prior to testing and then assayed using a quantitative PRRSV RT-PCR optimized for the oral fluid matrix.

Results: Of the 36 oral fluid samples collected on Study Day 8 (14 hours post placement of vaccinates), 23 (64%) samples were PRRSV qRT-PCR-positive. Of the 252 samples collected over the 7 days after Study Day 8, 195 (77%) tested positive. These data suggest that pen-based oral fluid sampling for PRRSV using an optimized PRRSV PCR assay is highly effective for the detection of virus in low prevalence populations.

Conclusions:

Data presented here corroborate previous reports suggesting that oral fluid sampling is a cost-effective alternative for PRRSV surveillance.

052

Accuracy of disk diffusion and broth microdilution by latent class analysis for *Escherichia Coli* and *Mannheimia Haemolytica* in feedlot cattle.

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Purpose: An understanding of error rates in antimicrobial susceptibility testing (AST) is crucial to establishing reliable estimates of antimicrobial drug resistance (AMR). Misclassification of any test is expected to some extent and the true state of disease or resistance is ultimately unknown. Bayesian analysis techniques are capable of modeling such uncertainty in classification for diagnostic tests. The objective of this study was to compare the accuracy of the disk diffusion and broth microdilution methods of testing antimicrobial susceptibility for surveillance of AMR in feedlot cattle.

Methods: Isolates of *E. coli* and *M. haemolytica* were tested for susceptibility to panels of antimicrobial drugs (AMDs) by standardized methodology for disk diffusion and broth microdilution. Latent class analysis was used to determine the proportions of correctly identified resistant and non-resistant isolates for each AST method. The AMDs shared between the AST panels and compared in analysis were ampicillin, ceftiofur, streptomycin, sulfonamide, tetracycline, and trimethoprim-sulfamethoxazole.

Results: A total of 2316 *E. coli* isolates from individual samples, 885 *E. coli* isolates from composite samples, and 783 *M. haemolytica* isolates were tested by both AST methods. Models for all organism and AMD combinations indicated that both AST methods classified relatively high proportions of non-resistance correctly. However, the correct classification of true resistance varied.

Conclusions: Disk diffusion and broth microdilution have comparable accuracy for most of the AMDs tested. Ceftiofur was not modeled well in this analysis due to extremely low occurrence of resistance in this population.

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053

Validity of the bovine TB gamma interferon assay on blood collected during exsanguination at slaughter.

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Purpose: Bovine tuberculosis (BTB) is of economic, regulatory, and zoonotic importance. It is caused by *Mycobacterium bovis*. The gamma-interferon (γ -IFN) assay is a blood-based test for BTB, which measures cell-mediated immunity to *M. bovis*. Integrating γ -IFN assay with the currently used visual inspection of carcasses in post-mortem slaughter surveillance could help detect more BTB herds. However, it is not known if a γ -IFN response, sufficient to produce a valid test, can be obtained using blood collected at exsanguination. We hypothesized that there is no change in γ -IFN interpretations between blood collected pre-slaughter and at exsanguination.

Methods: Sixteen cattle were experimentally sensitized with killed *M. bovis*, creating an immune response similar to that found in BTB infection. Four controls received mineral oil only. The γ -IFN assay was performed on blood samples collected pre-slaughter and at exsanguination.

Results: The probability that *M. bovis* sensitized cattle would remain γ -IFN positive on blood collected at exsanguination was 0.75 (95% CI 0.54, 0.88).

Using paired t-test analysis, there was a significant decrease in the mean ELISA optical density (OD) readings from pre-slaughter and at exsanguination ($p = 0.03$).

Conclusions: Although ELISA OD readings of individual cattle dropped at exsanguination, a change in the ELISA interpretation only occurred in animals that were borderline positive pre-slaughter. Potential factors responsible for the drop in γ -IFN response are being investigated. Our results suggest that the majority of cattle with a positive γ -IFN response pre-slaughter will remain positive at exsanguination. Therefore, γ -IFN assay may provide a useful tool for BTB surveillance at slaughter.

054

A Bayesian approach to estimate test parameters of 2 different tests for detection of *Mycobacterium avium* subsp. *paratuberculosis* fecal shedding in dairy cows.

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Purpose: The objective of this study was to estimate the probability of two tests (bacterial culture of feces and serum ELISA) to correctly identify cattle that shed high, low, and no fecal concentrations of *Mycobacterium avium* subsp. *paratuberculosis* (Map) into the environment.

Methods: The results of 12,957 simultaneous bacterial culture (HEY media) and serum ELISA (IDEXX) from 8 dairy herds enrolled in the Minnesota Johne's Disease Demonstration Herd Program over a 9 year period were used for this study. A Latent-class analysis using a Bayesian Markov-Chain Monte Carlo approach was used to estimate the conditional probabilities that test results indicate high, low, and no fecal shedding, given the true Map shedding status of the animal, $P(\text{test results} | \text{true status})$. This approach assumed that both test are imperfect, and they are conditionally independent. Shedding levels using bacterial culture were categorized as high with ≥ 50 colonies/slant, low with colonies/slant between 1 and 50, and no fecal shedding with no detectable colony growth on the slants. Likewise, levels for serum ELISA were established based on S/P values, with ≥ 1.0 (high), ≥ 0.25 and < 1.0 (low), and < 0.25 (negative). Informative prior Dirichlet distributions of the conditional probabilities were given by one of the co-authors (SJW).

Results: The probability of the serum ELISA to correctly identify high fecal shedders ($P(\text{High} | \text{High})$) was 69%, while the same probability for bacterial culture was 59%. The false negative rate for high fecal shedders, ($P(\text{No shedding} | \text{high})$) was 10% for serum ELISA and 3% for bacterial culture. Similarly, the false negative rate for low shedders ($P(\text{no shedding} | \text{low})$) was 78% and 73% for serum ELISA and bacterial culture, respectively. Test Specificities ($P(\text{No shedding} | \text{no shedding})$) were 99.8% for serum ELISA and 98.9% for bacterial culture.

Conclusions: This approach can be used to understand diagnostic test performance in diseases with multiple stages. In this case, these posterior conditional distributions improve the understanding of the bacterial culture and serum ELISA performance on the diagnosis of Map negative, infected and infectious animals.

055

Estimates of diagnostic test sensitivities and specificities: what confidence do we really have?

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Purpose: A multitude of methods for estimation of confidence intervals of proportions have been reported in the literature. Because of the discrete nature of the underlying distributions that these methods are based upon, the nominal 95% confidence (95CI) cannot be achieved exactly and can lead to poor coverage and inappropriate intervals. The objective of this communication is to illustrate the chaotic behavior of these methods.

Methods: Previously reported sensitivities and specificities of several diagnostic tests were retrieved to estimate 95CI with different methods. These methods included Wald, Wilson, Agresti-Coull, Jeffrey (or, Bayesian method), and Clopper-Pearson intervals (or exact interval). The exactitude of a 95CI can be estimated through the coverage probability, which represents the proportion where the interval contains the true value of interest.

Results: 51.9% of 27 samples from clinical pigs were reported as seroconverted to PCV2. 95CI upper bounds ranged from 57.7% (exact) to 62.1% (Wald) and coverage probability from 93.4% (Bayes) to 96.6% (exact). Test sensitivity for BVDV was reported to be 98.8% based on testing of 249 persistently infected cattle. The 95CI lower bound ranged from 96.4% (Agresti-Coull) to 97.4% (Wald). The coverage probability, ideally equal to 95%, ranged from 79.9% (Wald) to 98.9% (exact). The best-fitted coverage probability was observed with the Agresti-Coull method.

Conclusions: The erratic behavior of the coverage probability for 95CI was confirmed for estimates arising from small sample sizes; however, coverage probabilities were equally erratic in studies with large sample sizes and proportions close to 100%. Interestingly, the exact 95CI calculation, while relying on "exact" methods, does not actually provide an "exact" coverage probability. It is often described as conservative and leads to bad estimators. The Agresti-Coull interval presents a satisfactory compromise between computational requirements and the coverage probability. Ideally, the effects of coverage probability should be estimated and the most appropriate method chosen before reporting the results or use as priors in stochastic models.

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056

Comparison of crude and model-adjusted space-time scan statistic for food animal syndromic surveillance.

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Purpose: Space-time scan statistics are useful cluster detection methods for disease surveillance. However, there are limitations with Poisson and space-time permutation models. Poisson models for space-time scan statistics are limited at controlling for covariates and space-time permutation models are subject to population shift-bias when applied over long periods. The objective of this study was to compare the space-time clusters identified using three scan statistics with different abilities to control for covariates.

Methods: The spatial scan statistics were applied to monthly bovine liver parasite condemnation data from Ontario provincial abattoirs from 2001-2007. For the non-adjusted Poisson and space-time permutation space-time scans, the raw rates and total counts were used, respectively. For the adjusted Poisson space-time scans, a multi-level model was created that adjusted for economic and abattoir characteristics to generate the expected number of condemnations and the standardized morbidity ratios were analyzed.

Results: The number of significant space-time clusters varied among different space-time scans with two significant clusters for the unadjusted Poisson scan and four for the space-time scans using Poisson and space-time permutation models. While the geographical locations of clusters were similar between the latter two approaches, the time period of these space-time clusters rarely overlapped.

Conclusions: Variability in results among methods suggests that caution should be used in selecting space-time scan methods for abattoir surveillance. In this case, model adjusted scan statistics identified different space-time clusters than the other approaches. Ultimately, validation of different approaches with simulated or real outbreaks needs to be performed in selecting the appropriate statistical test for these data.

057

Assessment of passive laboratory data for use in an antimicrobial resistance surveillance system.

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Objective: The passive collection of data from clinical laboratories provides a less labour- and cost-intensive source of information for livestock disease surveillance compared to active data collection. The use of laboratory-based data may provide information about changes in the prevalence of pathogens over time, space and space-time that could support regional control measures. However, the primary objective of data collection at a clinical laboratory is to provide information to clinicians to assist in making diagnostic and treatment decisions for individuals, rather than to assess population health.

Therefore, data are primarily managed and stored with the objective of communicating individual results to veterinarians; they would be managed and stored differently if they were gathered primarily for surveillance and epidemiological purposes. These differences likely present challenges for rapid retrieval and analysis of data required by an efficient laboratory-based surveillance system. The objective of this study was to characterize the potential barriers to using diagnostic submission data from the Animal Health Laboratory at the University of Guelph for the development of a surveillance system for antimicrobial resistance (AMR) in pathogens isolated from Ontario livestock.

Methods: This objective was met by assessing differences, or “gaps” between AHL data quality and the data requirements for a successful AMR surveillance system.

Results: Gaps discovered included minimal farm demographic information, inconsistent recording of practice names and formatting in records for antimicrobial susceptibilities, changes to antimicrobial testing panels over time, missing information regarding breakpoint changes, and problems linking serotype information to specific submissions.

Conclusions: Identification of these gaps will direct interventions that may be applied to improve the utility of AHL data for surveillance purposes. Furthermore, this gap analysis may provide a framework for the development of other clinical laboratory database systems with links to passive surveillance systems.

058

Development of a longitudinal antimicrobial resistance and antimicrobial use surveillance program for the feedlot sector in Canada: Lessons Learned.

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Antimicrobial resistance (AMR) is considered one of the most important issues threatening public health in the new millennium, and some health experts have pointed to the use of antimicrobials in livestock as an area that should be carefully scrutinized. Therefore, it is important to be able to collect sound scientific data to evaluate the role of antimicrobial use (AMU) in food animal production on the development and spread of AMR. The objective of this project was to establish a framework for an AMR and AMU surveillance program in the Canadian feedlot sector. In 2007 a 3 year pilot project was initiated. It was designed to be a practical model for monitoring antimicrobial susceptibility in populations of feedlot cattle. This program was an important first step in the development of a national AMR and AMU surveillance program and targeted generic *E. coli*, and *Salmonella enterica* recovered from bovine fecal samples and *Mannheimia haemolytica* recovered from bovine nasal swabs. Sample collection, and laboratory methodologies were investigated to support an effective on-going program. The project generated AMR prevalence estimates in the organisms of interest, which is important industry-specific baseline data. It also provided individual and in-feed AMU data linked to the AMR data. Other research projects benefited from the project platform and were able to obtain baseline data on methicillin resistant *Staphylococcus aureus*, *Clostridium difficile* and *Campylobacter* species in feedlot cattle. In addition to the AMR and AMU data, the project demonstrated the importance of including organisms related to animal health and to public health, it resulted in the development of a PCR technique for identifying *M. haemolytica*, and it provided valuable insight into the value of, the cost of and the framework design for a national program.

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059

Measurement of low-quantity antibiotic resistance genes in agricultural samples: a hierarchical model for analysis of left-censored qPCR data. T. Boyer, University of Minnesota, Minneapolis, MN; R. Singer, University of Minnesota, Saint Paul, MN, *Email: boye0087@umn.edu*.

Purpose: One of the challenges of studying the relationship between agricultural use of antimicrobials and the spread of antimicrobial resistance has been the lack of quantitative methods available for measuring resistance. Quantitative real-time PCR (qPCR) applied to community DNA provides a method to measure the quantities of resistance genes in a sample. Although qPCR is highly sensitive, qPCR assays have limits of quantification that can present difficulties when studying genes that are present in very low quantities, often resulting in data that are left-censored. This can limit the utility of qPCR as a method to study emerging resistance. The objective of this study was to develop a hierarchical regression model that accounts for left-censored qPCR data and to apply the model to qPCR data from dairy cattle. We hypothesized that this approach would result in a more accurate description of the relationship between antibiotic use and resistance than other methods used to study resistance.

Methods: Fecal samples were collected from five cows that were treated with ceftiofur, a third-generation cephalosporin, and five untreated cows. Samples were collected prior to, during, and post-treatment. Community DNA was extracted from each sample and quantities of blaCMY-2, a cephalosporin resistance gene, were measured by qPCR. Computer simulations were used to assess the ability of the model to estimate regression parameters in the presence of censored data. The qPCR data were entered into the model to estimate the effect of ceftiofur treatment on blaCMY-2 quantities.

Results: The simulations showed that the regression parameters estimated by the model were less biased than parameters that were estimated when a fixed value was substituted for censored observations. The analysis of the samples from dairy cattle showed that blaCMY-2 quantities in both treated and untreated cattle rose during treatment and then decreased immediately after treatment.

Conclusions: The combination of qPCR and a hierarchical model that accounts for censoring provides a way to study the relationship between antibiotic use and resistance when the gene of interest is present in very low quantities.

060

Role of the environment in transmission of multi drug resistant (MDR) *Campylobacter* in antimicrobial free (ABF) pigs from farm to slaughter. M. Quintana-Hayashi, H. Pierce, S. Thakur, North Carolina State University, Raleigh, NC, *Email: mpquinta@ncsu.edu*.

Purpose: This longitudinal study was conducted to determine the role played by the environment in transmission of antimicrobial resistant *Campylobacter* to pigs reared in the ABF production system at farm and slaughter. **Methods:** Water, feed, swabs and soil samples (n=688) were collected from eight ABF farms along with 1,038 fecal samples from pigs at different production stages (Farrowing, Nursery, Finishing). Slaughter environmental samples (n=95) included lairage and truck, in addition to mesenteric lymph nodes, post evisceration and post chill carcass swabs (n=406) collected from the same pigs sampled on farm. A total of 851 isolates were tested for antimicrobial resistance to determine the minimum inhibitory concentration to nine antimicrobials by the broth microdilution method. **Results:** The environmental prevalence of *Campylobacter* was 20.7% compared to 72.1% in pigs. Water samples had the highest prevalence (36.3%) followed by floor swabs (29.4%), soil (20%) and lairage (19%). Other than four *Campylobacter jejuni* isolates from water and sows, rest of the isolates (n=933) were speciated as *Campylobacter coli*. A total of 32.3% (n=42) isolates from the environment were MDR (resistant to \geq three antimicrobials). The predominant pattern was the combination of azithromycin, erythromycin, telithromycin and clindamycin which was found in water, swabs, soil and lairage isolates. This pattern was also observed in pigs from farrowing (35.7%), nursery (50%) and finishing (15%), and in post evisceration (29.4%) and mesenteric lymph node isolates. The tetracycline resistant isolates encoded the *tet* (O) gene and the A2075G mutation of the 23S rRNA genes coded for erythromycin resistance. **Conclusion:** Based on phenotypic characterization we can conclude that the pig environment at ABF farm and slaughter plays an important role in the transmission of MDR *C. coli* to pigs.

061

The human-animal Brucellosis interface in Republic of Georgia: A descriptive study using a rapid assessment approach.

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Purpose: The purpose of this survey was to understand the disease ecology of Brucellosis in the Republic of Georgia based on animal management techniques, livestock movement, dairy production techniques, and dairy product distribution.

Methods: The investigation used a rapid assessment survey technique that used a semi-structured questionnaire, and convenience and purposive sampling until saturation.

Results: The survey data indicate that animal disease transmission is greatest on winter pastures, that the village can be the used as the unit of analysis for animal disease monitoring and that a closed herd system would be difficult due to seasonal pasturing and shared pastures. The greatest animal exposure is amongst male animal owners in the Azerbaijani, Georgian and Tatar ethnic groups. Sheep most commonly use seasonal pastures and are present at greater number than cattle on all pastures but especially on seasonal pastures despite being less commonly owned. As for dairy products, cheeses are not aged for long enough duration prior to consumption to prevent Brucellosis transmission despite local knowledge of this risk.

Conclusions: Disease control methods will have to be at the animal level rather than the food processing level due to resistance amongst the people of using boiled milk in cheese production. Animal interventions will be most successful on winter pastures and animal control policies on public seasonal pastures could be used to increase compliance of state mandated brucellosis control measures. Further, results of a case control study on human disease risk factors will be reviewed and an Agent-Based Model's preliminary results on the impact animal level interventions have on human disease incidence will be discussed.

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Bovine tuberculosis slaughter surveillance system in the USA and the ability to trace infected cattle back to the herd of origin.

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The detection of gross bovine tuberculosis (bTB) lesions in cattle at slaughter and the successful trace-back to the herd of origin is crucial to the detection of infected herds and for the success of the national bTB eradication program in the United States (USA). The objective of this study are to: 1) quantify the successful trace-back of bTB cases detected during slaughter inspection back to their herd of origin and 2) identify factors associated with the probability of identifying additional bTB infected cattle after bTB cases were detected at slaughter surveillance. Descriptive statistics and logistic regression analysis will be conducted to complete this study. During 2001-2009, 378 lesioned cattle were classified as bTB positive in the USA. Of these, 341 (90%) were in young (fed) cattle predominantly originating from feedlots, and 37 (10%) were culled adult beef and dairy cattle. Preliminary results show that of the 341 fed cattle, 262 (77%) originated from Mexico, where Mexican officials conducted epidemiologic investigations, 50 (15%) were of undeterminable origin and 27 (8%) originated from the USA with 6 (22%) of these successfully traceable to a herd in the USA. Of the 37 adult lesioned cattle, 33 (89%) originated from the USA with 23 (70%) successfully traceable to a domestic herd of origin. Domestic herds with at least one additional animal confirmed bTB infected in the herd were found in 3 (50%) out of the 6 fed cases and in 18 (78%) out of 23 adult cases. The factors impacting successful trace-backs of slaughter bTB cases to the herd of origin and the ability to identify additional bTB infected animals are currently under investigation. Our preliminary results highlight the importance of slaughter surveillance and trace-back of bTB cases in the overall efforts to eradicate bTB in the USA.

063

A meta-analysis of the effects of feeding yeast culture produced by anaerobic fermentation of *Saccharomyces cerevisiae*, on milk production in lactating dairy cows.

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Purpose: This study evaluated the effectiveness of feeding Yeast Culture (YC) produced by anaerobic fermentation of *Saccharomyces cerevisiae*, and manufactured by Diamond V, on milk production and milk components in lactating dairy cows. A subgroup analysis was performed to determine the change in meta-analysis outcome for peer reviewed journal random controlled trials (RTC) versus including published abstracts, Diamond V reports, and study designs of cross over trials (CO) and Latin Square (LS) designs.

Methods:

A complete literature search was performed. The criteria for inclusion were, only lactating dairy cows, fed Diamond V's YC products, had a contemporary negative control, and be an RCT, CO or LS. Our search identified, 15 peer reviewed journal articles with 28 comparisons, 7 published abstracts with 13 comparisons and 18 Diamond V reports containing 26 comparisons. A meta-analysis was performed utilizing Comprehensive Meta Analysis (Version 2.2.050).

Results: The meta-analysis of published journal RCT found these weighted mean differences; milk yield (MY) 1.21 kg (95% CI: 0.54, 1.88; $P < 0.001$), dry matter intake early lactation (DMIe) 0.56 kg (95% CI: 0.17, 0.95; $P = 0.005$), dry matter intake mid and late lactation (DMIml) -0.11 kg (95% CI: -0.43, 0.21; $P = 0.49$), percent fat (%F) 0.063% (95% CI: -0.17, 0.3; $P = 0.6$), percent protein (%P) 0.01 (95% CI: -0.11, 0.14; $P = 0.14$), and 3.5% adjusted fat corrected milk (3.5% FCM) 1.75 kg (95% CI: 1.19, 2.3; $P < 0.001$). The meta-analysis utilizing all comparisons from journals, abstracts and reports yielded, MY 0.93 kg (95% CI: 0.68, 1.18; $P < 0.001$), (DMIe) 0.34 kg (95% CI: 0.1, 0.58; $P = 0.006$), (DMIml) 0.095 kg (95% CI: -0.16, 0.35, $P = 0.47$), %F 0.053% (95% CI: -0.03, 0.13, $P = 0.19$), %P 0.05%, (95% CI: -0.09, 0.2; $P = 0.48$) and 3.5% FCM 1.12 kg (95% CI: 0.81, 1.43; $P < 0.001$).

Conclusions: The results of this meta-analysis showed the use yeast culture produced by anaerobic fermentation process can improve milk production and milk components in lactating dairy cows. Further the use of journal abstracts and non-peer reviewed reports produced similar results to an analysis using only peer reviewed RCT, but tended to produce results closer to the null.

064

Correspondence analysis-a method of assessing relationships between categorical variables.

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Many studies gather data that are categorical in nature. Multiple Correspondence analyses (MCA) is a non-parametric technique that allows visualization of the association between multiple categorical variable. MCA can be used as a screening method employed upon a large number of categorical variables without definitively assigning dependent or independent status. Using data from the National Animal Health Monitoring System's (NAHMS) Swine 2006 study, 281 farm-level variables were selected that were hypothesized to be associated with the level of *Streptococcus suis* on farms. These variables were regressed in a univariate fashion with an outcome of *S. suis* in a farm's nursery pigs and entered into a MCA to detect associations not only with the tentative outcome but among one another. Variables that showed association with *S. suis* in the univariate regressions or MCA (in the Correspondence Map or related chi square/inertia contribution output tables for the latter) were then separately regressed in RLOGIST in SUDAAN against *S. suis* status using weighted data. It was more time effective to assess potential associations and collinearity using MCA rather than performing multiple univariate regressions. The potential for sporadic association with so many variables was also reduced by using MCA. However, although MCA allows visualization of associations it does not always indicate the direction or strength of that association with the outcome variable.

065

Using mixed treatment comparisons meta-analysis to compare interventions for which no head-to-head comparison exists: Treatments for bovine respiratory disease.

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The purpose of this study was to evaluate the use of mixed treatment comparison methodologies to compare interventions in veterinary science. In veterinary science the number of interventions studies published is small therefore, although practitioners may prefer to rely upon the result of

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published randomized clinical trials, it is common that no published trials may exist for many comparisons of interest. In human medicine, similar situations arise, when multiple interventions are available but no direct comparisons are available, for example, trials may exist that directly compare Treatment A to Treatment B, and Treatment A to Treatment C, but no head-to-head comparisons of Treatment B to Treatment C exist. In human clinical medicine, it is increasingly common to make such indirect comparisons of Treatment B to Treatment C using Bayesian Network Analysis. In this study we evaluate using Bayesian Network Analysis to make indirect comparison of interventions for bovine respiratory disease. The population of interest was antibiotic therapies for bovine respiratory disease in North American feedlot cattle. Data from the meta-analysis were obtained from studies published in the peer-reviewed literature, conference proceedings and the FDA CVM Freedom of Information (FOI) Drug Summaries were used in the study. Information extracted from each study included all treatments assessed including controls (several studies used multiple trial arm designs), the study sponsor, if blinding and randomization were included in the description of study execution, number of animals in each group and number of animals with a BRD relapse with 14 to 28 days post treatment. As well as ranking the treatments based on direct and indirect comparisons, the results assessed the consistency of direct and indirect comparisons and the potential impact of study design bias and sponsorship bias on the comparisons.

068

Diagnostic sampling strategies for virulent ovine footrot: simulating detection of *Dichelobacter nodosus* serogroups for bivalent vaccine formulation
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Dichelobacter nodosus is a slow-growing anaerobic bacterium that is the causative agent of virulent ovine footrot. Vaccination targeted at up to two specific serogroups can eliminate those serogroups from infected flocks, but requires identification of serogroups present in infected flocks. Serogroups can be identified using slide agglutination or polymerase chain reaction (PCR) methods. The objectives of this project were to use stochastic simulation modeling to estimate the efficacy of sampling strategies encompassing 5-40 sheep per flock and 2-4 colonies per sheep, and to compare efficacies based on slide agglutination or multiplex PCR test results. Foot swabs collected from sheep in 12 flocks were used as the basis for a sampling strategy simulation model. None of the evaluated sampling strategies identified the two most common serogroups in the flock, or all serogroups present in the flock, in 95% of iterations. A simulated sample of 22 sheep/flock and 2 colonies/sheep resulted in a simulated vaccine that protected 95% of the sheep that could be protected by a single bivalent vaccine, while a sample of 24 sheep/flock and 2 colonies/sheep resulted in a series of simulated bivalent vaccines that protected 95% of diseased infected sheep. The difference in outcome was due to the distribution and frequency of serogroups within certain flocks where some serogroups were uncommon and others dominant. Evaluating efficacy based on the expected effect on the flock may be more useful than one which seeks to determine the most common serogroups.

070

Retrospective study of HPAI outbreaks in domestic poultry in mainland China from 2004 to 2009.

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Purpose: Since 2004, China reported 39 human cases of HPAI, which accounted for 7.8% of all human cases in the world. Outbreaks in China also affected more than 360 million domestic poultry. Little was known about epidemiology of HPAI in mainland China, either before or after compulsory vaccination was implemented. The objective of this cross-sectional study was to describe epidemiological characteristics of HPAI in mainland China using available official records from January 2004 to September 2009.

Methods: Data on domestic poultry demography, outbreaks, and surveys were retrieved and validated. Descriptive statistics were used to describe population at risk. Records of outbreaks and virological and serological surveys were investigated according to species, location and time.

Results: The statistics showed that the total poultry number and scale of poultry farming were in an upward trend, while poultry distribution had unique regional characteristics. Out of 107 HPAI outbreak reports, farms with more than one poultry species comingling (39.4%) and with less than 10,000 heads (91.8%) were the most affected. More outbreaks were reported in the traditional waterfowl breeding region in the south, such as Guangdong, Hubei and Hunan provinces. Relatively higher number of outbreaks was reported in February and October prior to 2006. From 2006 to 2009, relatively higher number of outbreaks was recorded in May and June. The analysis of survey data showed the same trends as those seen in the outbreaks.

Conclusions: In this study, all the data used were from official database or publication. Further study would be undertaken to analyze the relationship between HPAI situation and implemented control measures, including vaccination

071

Public health and zoonoses: A survey of Canadian Public Health personnel regarding knowledge, practice and education.

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Purpose: Zoonoses are a major public health concern. In Canada, investigations of zoonotic disease incidents are often conducted by Public Health Inspectors (PHIs). Little is known about PHIs' knowledge of transmission of zoonotic pathogens, their perceptions of zoonotic disease importance or their education regarding zoonotic diseases. The objective of this study was therefore to assess the knowledge, perceptions and education of Canadian PHIs regarding zoonotic diseases. **Methods:** Data was collected from December 2008-January 2009 using an internet based survey distributed to members of the Canadian Institute of Public Health Inspectors listserv, and described using frequencies.

Results: Responses were received from 229 PHIs in four provinces, with a response rate of approximately 20%. The majority of respondents reported at least 10 years of experience in the public health sector, 80% were in frontline positions, and 62% were routinely involved in investigations of infectious diseases. Two-thirds believed that the importance of zoonotic disease with regards to public health would increase in the next 5 years. While most respondents were able to correctly identify animals capable of directly transmitting common zoonotic pathogens, there were gaps in knowledge, particularly with regard to rabies, tick-borne illnesses and transmission of gastro-intestinal pathogens by companion animals. PHIs tended to feel that their training on zoonotic diseases prior to working at PHIs was deficient in some areas, or left some room for improvement. Less than one third of PHIs received ongoing continuing education regarding zoonotic diseases, and of those that did, nearly two-thirds rated the quantity and quality as only fair. PHIs expressed interest in methods of continuing education that required less time commitment such as seminars and e-mail bulletins.

Conclusions: The results of the survey indicate that there are gaps in PHIs' knowledge of zoonotic diseases, and a need for improvement in both the quantity and quality of continuing education for PHIs on zoonotic diseases.

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Carriage of methicillin-resistant *Staphylococcus aureus* (MRSA) among selected Michigan 4-H club members and their backyard-raised pigs
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Purpose: In order to address the concern regarding methicillin-resistant *Staphylococcus aureus* (MRSA) colonization among pigs and their handlers, a two-part study was conducted among selected members of pig-raising 4-H club members in selected Michigan counties.

Methods: The first study involved assessing the carriage of nasal MRSA and the coagulase-negative staphylococci (CNS) among 4-H volunteers with extensive pig contacts (n=114). The second phase of the study involved assessing the nasal MRSA carriage of matched owner-pig pairs (n=50 pairs) along with a limited unmatched pig (n=3) and human (n=4) samples. Confirmed MRSA isolates were further characterized by pulsed-field gel electrophoresis (PFGE), SCCmec typing, multiple locus strain typing (MLST), and identification of the agr and PVL genes.

Results: For the first phase of the study, MRSA was recovered from one adult male with sheep contact (1/114 or <1%). This MRSA isolate was a USA 100 strain, SCCmec IV, ST 5 and PVL negative. For the second phase of the study, no paired owner-pig was found positive, however, 1/54 (1.9%) of the human samples and 2/53 (3.8%) of the pigs were found to be carrying MRSA. The human isolate had a PFGE pattern not previously described by CDC. It has SCCmec type IVb, ST8, agr I and was PVL negative. Both pig isolates had identical PFGE pattern which was also not previously described by CDC; both were classified as SCCmec type III, ST 5, agr II and PVL negative.

Conclusions: These MRSA findings in pigs indicate that these were more likely to be spill-overs from human carriage rather than naturally maintained in the pig population. Both phases of the study also indicate that the local backyard swine raisers from selected Michigan counties currently have MRSA carriage rates similar to that of the general U.S. population, suggesting that backyard pig-raising did not increase their risk for contracting MRSA. This may, however, eventually change given the continuing movement of pigs and people within and around Michigan, and the continuing genomic evolution of circulating MRSA among people and livestock.

073

Environmental role in the transmission of multidrug resistant (MDR) Salmonella to conventional pigs at different stage of production at farm and slaughter.

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Purpose: There is little information on the role played by farm and slaughter environment in transmission of Salmonella at various stages of pork production. The aim of this longitudinal study was to determine the prevalence and molecular characterization of MDR Salmonella in conventional pigs at farm and slaughter and in their environment.

Methods: Samples from a cohort of 30 pigs per farm and their environment (feed, water, soil, lagoon, truck and floor swabs) were collected in 10 conventional farms at different stages on farm (farrowing, nursery, finishing) and slaughter (post-evisceration, post-chill, mesenteric lymph nodes-MLN). A total of 1570 fecal and 1245 environmental samples were collected at farm. In addition, 620 slaughter and 40 lairage and truck samples each were collected at slaughter. Salmonella was characterized for their antimicrobial resistance profile, resistance genes, class I integrons. Genotypic relationships among Salmonella isolated from the pigs and their environment were determined by Pulsed-field gel electrophoresis (PFGE).

Results: The overall prevalence of Salmonella at farm in pigs was 4% (n=63) and 11% (n=141) in the environment. Salmonella was isolated from 21% MLN and 3% post-evisceration swabs. The highest prevalence of Salmonella were detected from the slaughter lairage (37%; n=15) and truck (30%; n=12). The highest frequency of resistance was detected to tetracycline in pigs (86%) and environment (80%). MDR (resistance to 3 anti-microbials) were detected in 29% (n=231) 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, strA, strB, aadA1, aadA2, tetG 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 that the environment plays an important role in transmission of antimicrobial Salmonella to the conventional pigs at different stages of production.

074

Prevalence of zoonotic pathogens in the Canadian pork chain: evidence from a scoping review.

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Purpose: In a number of areas of policy and decision making the scientific literature is reviewed for availability of evidence to support decisions. Systematic reviews (SR) provide for the synthesis of published evidence on a specific question. Originally developed in the area of clinical trials, their use has been explored to synthesize evidence on diagnostic tests and prevalence. However, SR are demanding of resources and may not always be warranted or possible. As an alternative, scoping reviews have been used in other fields to explore evidence available on a research topic using a structured approach in a more flexible and efficient format. Scoping reviews are suited to more general questions than SR and a map of the evidence available can be created, clearly identifying where focused evidence synthesis is possible and where gaps in the evidence exist. Scoping reviews employ many of the same characteristics of a SR, including transparency and replicability. Available literature is screened for relevance and categorized for criteria relative to the research question. In this study, a scoping review of the prevalence of 15 zoonotic pathogens in pigs and pork on farm, at slaughter and in retail in Canada was conducted.

Methods: A replicable search strategy, along with structured and transparent relevance screening tools were used to identify and categorize potentially relevant abstracts by pathogen, population, study design, and sample size.

Results: Over 30,000 references were identified, screened for relevance and categorized by two independent reviewers. One hundred and one references reported the detection of one or more pathogens at farm (52), slaughter (38) and/or retail (17) in Canada since 1990. Fifteen references reported sampling for *Salmonella* at farm or slaughter, compared to 6 reporting sampling for *Campylobacter* and 3 reporting sampling for *Toxoplasma*. References reporting sampling raw pork at retail tested for *Campylobacter* (2), *Clostridium* (1), *E. coli* (6), *Listeria* (3), *Salmonella* (7), *Staphylococcus* (1) and *Yersinia* (4).

Conclusions: Numerous study designs were employed and sample sizes varied. The published data available for a number of the pathogens was limited.

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Food Systems Veterinary Medicine for the 21st Century.

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For good or for ill, food production is consolidating and intensifying. Today one decision may affect one carcass which may have a global impact (e.g. international trade bans due to one tissue residue violation, or large-scale disease outbreak). In three months one egg producer can supply a half billion potentially contaminated eggs. Therefore producers and their consulting veterinarians have influence on a much larger scope of animals, consumers, and the environment. No matter where in the food supply chain a veterinarian is working, they must understand the implications of decisions throughout the food, environment and public health systems.

Needed is a new discipline. For a few decades thoughtful researchers such as members of the CRWAD have expanded the disciplinary focus of animal research to include epidemiology, economics, and risk assessment, but application of these disciplines seems to be for the highly trained and for selected problems. This new discipline needs to "automatically" bring together the essential other ones, while providing a new perspective or framework for dynamic problem solving.

Systems engineering (SE) is this discipline. The systems approach is a holistic view of the elements and processes working together to produce a desired result or change an undesired system output. SE can help us understand the various interacting systems such as infectious agent, environment, animal hosts, food harvesting, etc. SE has a methodology and standards of practice (www.incose.org). The challenge is for us now is to adapt and apply those standards to animal health research and incorporate them in our practice and teaching.

076

Comparison of *Salmonella* testing results among NAHMS Dairy Studies conducted in 2007, 2002, and 1996.

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Purpose: The objective of this analysis is to report results from the NAHMS Dairy 2007 Study and to compare results on the occurrence of *Salmonella* in NAHMS dairy studies conducted in 1996, 2002, and 2007.

Methods: Each of the NAHMS dairy studies involved collecting fecal samples from 35-50 cows on approximately 100 dairy operations across the U.S. *Salmonella* culture methods were similar across all three studies. Each NAHMS dairy study was conducted in states that represented approximately 80% of U.S. dairy operations and dairy cows.

Results: In the 2007 study, individual cow fecal samples were taken on 121 operations, and 48 operations (39.7%) had a least one *Salmonella*-positive healthy cow, with 523 of 3,804 (13.7%) of the healthy cows sampled being *Salmonella*-positive. The two most common serotypes identified in cows and on operations in the 2007 study were S. Cerro and S. Kentucky, respectively. In comparison, in the NAHMS Dairy 1996 Study, there were 18/90 operations (20.0%) with at least one *Salmonella*-positive healthy cow with 194/3,585 healthy cows (5.4%) being *Salmonella*-positive. In the NAHMS Dairy 2002 Study, there were 30/97 operations (30.9%) with at least one *Salmonella*-positive healthy cow with and 259/3,645 of the healthy cows (7.1%) were *Salmonella*-positive. A logistic regression model controlling for effects of herd was used in a preliminary assessment to compare animal-level results among the three NAHMS studies, using year as an explanatory variable and adjusting for herd size and region. Cows were significantly more likely to be positive in 2007 compared to 1996 (OR 2.5; 95% CI 1.1, 5.8). A similar analysis using logistic regression was performed using herd status (*Salmonella* positive or negative) as the outcome and year as an explanatory variable. Operations were significantly more likely to be positive in 2007 compared to 1996 (OR 2.5; 95% CI 2.1, 3.0).

Conclusions: The NAHMS dairy studies were not designed to provide a nationally representative estimate of *Salmonella* prevalence on U.S. dairies, but they do examine dairies across the U.S. Results suggest that *Salmonella* occurrence is at a higher level in the most recent NAHMS study compared to previous studies.

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Dose- and serotype- dependent dynamics of fecal shedding and immune response post *Salmonella* inoculation in pigs.

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Purpose: The objective was to quantify transition of pigs through the states of *Salmonella* fecal shedding and immune response post *Salmonella* inoculation as affected by the challenge dose and serotype using previously published experimental data.

Methods: A multistate modeling approach was applied under the assumption that a continuous time Markov process determines the transition times between the states. The multistate model developed for shedding consists of four transient states ("latency", "continuous shedding", "intermittent non-shedding", and "intermittent shedding") and one absorbing state representing clearance of *Salmonella* from feces ("recovery"). Pigs could progress to the recovery state from the latency, and the continuous and intermittent shedding states. The multistate model developed for a pig's immune response consists of two transient states representing response below and above the seroconversion level. The effects of dose [low (0.65×10^6 CFU) vs. high (0.65×10^9 CFU)] and serotype [feed-associated (*Salmonella* Yoruba and *Salmonella* Cubana) vs. "classical" pig (*Salmonella* Typhimurium and *Salmonella* Derby)] on the models' transition intensities were evaluated using a proportional intensities model.

Results: Analysis indicated statistically significant effects of the challenge dose and serotype on the dynamics of shedding and immune response. Pigs challenged with the high dose started shedding the pathogen sooner and continued shedding for a longer period of time as part of the continuous and intermittent shedding states, than pigs challenged with the low dose. Pigs challenged with the feed-associated serotypes transitioned to recovery sooner than pigs challenged with the "classical" pig serotypes. With respect to the immune response, pigs challenged with the "classical" pig serotypes seroconverted faster than those challenged with the feed-associated serotypes, while pigs challenged with the high dose remained above the seroconversion level longer than pigs challenged with the low dose.

Conclusions: The findings of this study will aid efforts to detect and control *Salmonella* in the pig reservoir, thereby decreasing the risk of human infection.

078

Molecular characterization of multidrug resistant (MDR) *Salmonella* Typhimurium from swine and human origin by phage types, resistance genes, integrons and PFGE.

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Purpose: The MDR *Salmonella* Typhimurium DT104 is one of the most important foodborne pathogen transmitted to humans worldwide. The objective of this study was to detect and analyze the antimicrobial resistant genes, integrons and clonal relationship between temporally and spatially related *Salmonella* isolates from pigs and human origin. **Methods:** A total of 1684 *Salmonella* isolates (pigs n=783; human n=901) was collected from various regions of North Carolina and characterized for their antimicrobial resistance profile to a panel of 15 antimicrobials. A total of 48 MDR *Salmonella* isolates from swine (n=24) and human (n=24) were further characterized based on similar serotype and MDR patterns to detect the presence of resistance genes, class I integrons and phage typing by PCR. Pulsed-field gel electrophoresis (PFGE) was used to determine the genetic relationships among the *Salmonella* isolates of human and pigs. **Results:** A wide spectrum of antimicrobial resistance was observed among swine and human *Salmonella* isolates. Multidrug resistance (resistance to ≥ 3 antimicrobials) were detected in 29.5% (n=231) of pig isolates and 12% (n=110) of the human clinical isolates. Within these MDR isolates, 18% (human, n=20; pigs, n=42) exhibited resistance to third generation cephalosporins including ceftriaxone and ceftiofur, which tested positive for *bla*_{CMY-2} by PCR. *S. Typhimurium* phage type DT 104 exhibited a distinct penta resistant profile characterized by resistance to ampicillin, chloramphenicol, streptomycin, sulfisoxazole and tetracycline (AMP CHL FIS STR TET) in 6% (n=57) of human and 3% (n=42) pigs isolates. Isolates with this resistance pattern were positive for *bla*_{TEM}, *cmlA*, *aadA1*, *aadA2*, *tetG* resistance genes and carried class 1 integrons of 1 and 1.2kb size. The PFGE patterns of pig isolates were different from human clinical isolates. **Conclusions:** There was no evidence that the isolates from pigs were genotypically linked with human clinical *S. Typhimurium* DT 104 in our study.

079

Longitudinal study of Salmonella shedding in finishing pigs in a multi-site production system.

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Purpose: The objective of this study was to describe the shedding pattern of Salmonella in finishing pigs, as well to quantify the Salmonella load. **Methods:** A longitudinal study has been conducted in 3 sites of a multi-site farrow-to-finish production system. For each site 6 consecutive cohorts were included in the study. In each cohort, 50 individual pig fecal samples were collected and cultured every 2 weeks for 16 weeks. Further, Real Time Quantitative PCR (QPCR) was performed in a subset of the culture negative samples and in all the culture positive samples. **Results:** At the time of submission 12 cohorts are complete. Salmonella was cultured from 349 (9%) of 4539 individual fecal samples. Overall incidence of Salmonella was 24.8% (149/600 pigs). The proportion of positive samples decreased by pig age, from 17% (10 week old) to 4% (24-26 week old). Of positive pigs, 83% were culture positive 4 or less times during the sampling period. At the present, all the Salmonella culture negative samples that were submitted to QPCR were negative. Of culture positive samples, 18% were detected by QPCR. Among the culture positive samples the Salmonella concentration ranged from 2.5 to 6.2 log₁₀ copies number per gram. **Conclusions:** The results suggest that point estimates of Salmonella prevalence do not determine accurately Salmonella status of the finishing pigs. The Salmonella status depends on sampling time, diagnostic test and load. These preliminary descriptive data represent the most intensive sampling to describe the epidemiology of Salmonella in swine to date.

080

Salmonella enterica in swine production: Temporal and spatial diversity based on amplified fragment length polymorphism method.

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Purpose: The purpose of this study is twofold: 1) to determine the genotypic diversity of *Salmonella* from hierarchical samples; and 2) to determine the epidemiological relatedness of *Salmonella* isolated from production chain, farms and slaughterhouses.

Methods: Each of 27 farms in U.S. Midwest was visited for four lots during September 2006 to February 2009. For each lot, 30 farm fecal samples from individual live pigs on farm and 30 mesenteric lymph node samples at slaughter were collected and matched on lot level. A total of 259 *Salmonella enterica* isolates from swine were identified using cultural method. All the isolates were genotyped using a high-resolution fingerprinting approach: amplified fragment length polymorphism (AFLP). To analyze genetic differentiation between farms, between lots and between samples, we performed pairwise nested analysis of molecular variance (AMOVA). A permutation test was conducted to determine the relatedness of *Salmonella* from farm feces and mesenteric lymph nodes with 4999 permutations.

Results: AFLP was able to completely separate the isolates. For the farm fecal samples, the "among farms" variance component was 27.22%, "among lots" was 7.18%, and the "among samples" variance component was 65.60%. The AMOVA test results suggested that the among farm and sample variance components were significant. The genetic distance between isolates from farms and slaughterhouses matching on farm or lot was tested using a permutation test, indicating the genetic distance between isolates of the same sources was not significant smaller.

Conclusions: The very low portion of variance explained by lots might suggest multiple visits are not necessary better describe the *Salmonella* AFLP variation on swine farms. There was no statistical evidence that the genetic distance of farm and slaughterhouse isolates from the same farms or lots are more related than from different farms or lots.

081

Variation in RAPD-PCR patterns is not attributable to genetic differences in Salmonella Enteritidis.

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Salmonella Enteritidis (SE) is the leading cause of gastroenteritis associated with consumption of contaminated poultry meat and eggs. Because PFGE has limited utility in distinguishing between clonal SE isolates, RAPD-PCR has been recommended as an alternative molecular fingerprinting tool. The objective of this study was to determine if increasing PCR stringency would reduce the amount of randomness in RAPD DNA patterns. Three different methods for DNA template preparation were evaluated, and were found to provide comparable results with respect to the similarities that were observed with repeated analyses of the same SE isolates (n = 18, P = 0.91). An *in silico* PCR was performed to predict amplification products that would be expected when using three different RAPD typing primers (1247, 1283, and OPA4) and to determine whether any of the primers would be more likely to amplify variable regions. A comparison of within- and between-isolate similarities was performed using RAPD- primer 1247, which was predicted by *in silico* analysis to yield the most variable size range of amplicons, especially from variable regions in the SE genome. Although the median within-isolate similarity (76.0%) was significantly greater than the median between-isolate similarity (66.7%; P = 0.001), even duplicate runs of the same SE isolates produced RAPD patterns that ranged in similarity between 61.5% and 100%. In conclusion, the reproducibility of RAPD-PCR was not sufficient to reliably

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distinguish between related and unrelated SE isolates. If RAPD-PCR is used to evaluate SE strain differences, duplicate samples of the same isolates must be analyzed to assess method variability.

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Class 1 integrons among multidrug resistant *Salmonella enterica* serovars from food animals, production environment and humans from two geographic locations.

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Purpose: This study was designed to identify and characterize class 1 integrons among multidrug resistant (MDR) *Salmonella* serovars. Methods: Isolates were recovered from two different geographic locations US (n=76) and Ethiopian origin (n=33) consisting of isolates from swine (n=58), swine production environment (n=28), chickens (n=3), camels (n=6), sheep (n=2), goats (n=1), bovine (n=4) and humans (n=7). A total of 109 epidemiologically unrelated *Salmonella* isolates of comprising 15 different serovars were characterized phenotypically (antimicrobial resistance, R-types) and genotypically (PCR for integrons).

Results: Class 1 integrons of different sizes were detected in 83 of 109 (76.1%) MDR *Salmonella* isolates. Isolates with 1.0 Kb single integron included Anatum (n=9), Derby (n=2), Inverness (n=1), Kentucky (n=9), Muenchen (n=1), Newport (n=2), Schwarzgrund (n=1), and Typhimurium (n=1) and others (n=8); isolates with two integrons of 1.0 and 1.2.kb sizes included Derby (n=1), Newport (n=1), Typhimurium DT104 (n=2), Copenhagen (n=1) and other isolates (n=11). We found some serovars with single integron of larger than 2.0 kb: Blockely (n=4), Mbandaka (n=2), Newport (n=1), Typhimurium (n=1) while the remaining isolates (Kiambu, London, etc) had amplicon sizes below 1000bp. A 2.5kb integron among MDR serovar Blockely with R-type CipKmNiNyNfStTe (n=4) was detected from porcine samples in Ethiopia but not the US.

Conclusions: While similar integron sizes and numbers were detected in the two geographic locations, unique integrons distinct to a geographic location were also detected. Further analysis of the isolates to determine the DNA sequences of resistance gene cassettes in the variable region of the integrons is currently underway. The findings so far show that class 1 integrons of varying sizes are common among MDR *Salmonella* regardless of geographic locations.

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Salmonella vaccination of broiler breeder pullets reduces *Salmonella* prevalences and loads in broiler chickens.

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Purpose: The objective of this study was to evaluate the effect of *Salmonella* vaccination in broiler breeder pullets on *Salmonella* prevalences and loads in breeder and broiler chicken flocks.

Methods: Pullets placed on six commercial breeder farms were vaccinated with an autogenous killed *Salmonella* bacterin made from isolates of S. Typhimurium, S. Enteritidis, and S. Kentucky. Unvaccinated pullets placed on six additional farms served as controls. Eggs from vaccinated and unvaccinated breeder flocks were kept separated in the hatchery, and the resulting chicks were used to populate 58 commercial broiler flocks using a pair-matched design. Four drag swabs and four boot socks were collected from each breeder flock at 35 and 45 weeks of age, and from broiler flocks one week prior to processing. In addition, 20 rehang birds were collected from broiler flocks at processing for the extraction of cecal samples.

Results: There was no significant difference between vaccinated and unvaccinated breeder flocks with respect to *Salmonella* prevalences or loads, but there was a significant difference between broiler flocks. After adjusting for sample type and clustering at the farm level using a population-averaged GEE logistic model, the odds of detecting *Salmonella* in samples collected from broiler flocks that were the progeny of vaccinated breeders were 62% lower (OR = 0.38; 95% CI = 0.21, 0.68) than in flocks that were populated with the progeny of unvaccinated breeders. In addition, the mean load of culture-positive samples was 0.3 log₁₀ MPN/sample lower in vaccinated than in unvaccinated broiler flocks, corresponding to a 50% decrease in *Salmonella* loads (P = 0.004).

Conclusions: Vaccination of broiler breeder pullets reduced *Salmonella* prevalences and loads in their broiler progeny, but did not significantly decrease *Salmonella* in the breeder farm environment.

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Effect of vegetable protein meal use in poultry feed on colonization and shedding of *Salmonella heidelberg* in broiler birds.

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Purpose: The objective of this experiment was to determine the effect of vegetable protein meals (soybean and canola) in poultry feed on colonization and shedding of *Salmonella heidelberg* in broiler birds over a 42-day period compared to broiler birds fed animal protein meal (meat and bone).

Methods: One-day old chicks were assigned randomly to 4 different dietary treatments (n=360 birds per treatment) with 6 replicates per treatment, 60 birds per replication. Three all vegetable protein meal diets and one commercial diet containing animal protein meal were used in the study. Half of the birds (n=30) per pen were challenged with nalidixic acid-resistant (NA) *S. heidelberg* on day one (called seeders), and the remaining un-challenged birds were called contacts. Drag swabs were collected from all pens on days 0 (prior to placement), 14, and 42. Ceca samples were collected from 20 birds per pen (10 seeders and 10 contacts) on day 42. Drag swabs and ceca samples were examined for NA- *S. heidelberg* using enrichment and enumeration/enrichment, respectively.

Results: All drag swabs were negative on day 0, but positive for *S. heidelberg* on both days 14 and 42. Within seeder and contact birds, there was no statistically significance difference in NA- *S. heidelberg* concentration (cfu/g of ceca) nor in proportions of positive ceca among the treatment groups.

Conclusions: The results obtained herein suggests that all vegetable protein meal diets did not significantly reduced the environmental contamination with *S. heidelberg* nor did it reduce the concentration and proportion of positive *S. heidelberg* in contact and seeder birds compared to commercial diet containing animal protein meal.

085

Movement of Bacteria in the Environment via the Lesser Mealworm, *Alphitobius diaperinus* (Coleoptera: Tenebrionidae).

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Purpose: In order to devise realistic management practices to mitigate the inadvertent dissemination of pathogenic bacteria around animal production facilities, it is important to understand the sources of reservoir populations. The lesser mealworm, *Alphitobius diaperinus* (Panzer), is a common arthropod pest in poultry litter that carries pathogens affecting human and animal health.

Methods: In a series of experiments using marker bacteria, we have characterized this pest as a reservoir of *Salmonella* dispersal.

Results: We demonstrated *Salmonella* are easily acquired by this insect from contaminated poultry litter sources and harbored internally. Subsequently, internally harbored bacteria can quickly contaminate the environment in which the beetle resides. The gut transit time of *Salmonella* was determined to be 2 to 3 hrs for both larvae and adults. In addition, we established that recently acquired *Salmonella* can reside within the gut for more than a week.

Earlier studies demonstrated that both the larval and adult stages could acquire bacteria from external sources and that conjugal transfer of antimicrobial resistance plasmids between the bacteria could occur within their gut.

Conclusions: Because of the mobility, voracious feeding habits, and migration potential of these beetles, it is likely that they become an active source facilitating the dissemination of bacteria and antibiotic resistance genes around animal production facilities and into the adjacent environment. Current farm practices perpetuate infestations and contribute to the dispersal of these beetles into the surrounding environment along with any bacteria that they carry. These studies serve as a model demonstrating bacterial load thresholds and movement of bacteria by these insects.

086

Characterization of the bacteriophage Felix O1 endolysin.

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Purpose: Bacteriophage Felix O1 is a bacteria-infecting virus specific for and lethal to most members of the genus *Salmonella*, a common cause of foodborne illness. Previous studies conducted by our lab have demonstrated that whole Felix O1 can be a successful control measure when applied to chicken frankfurter samples artificially contaminated with *Salmonella* DT104. The aim of this study is to determine the efficacy of the purified endolysin, a lytic enzyme produced by Felix O1, as an application for control of *Salmonella* in raw and ready-to-eat poultry products.

Methods: The lysis gene *lys*, identified in a previous study, was amplified by PCR and cloned into the expression vector pRSET A to create pRSETA/Lys1. The recombinant plasmid was transformed into chemically competent Mach 1 *E. coli* for storage and propagation. The endolysin was overexpressed in chemically competent BL21 Star (DE3) pLysS *E. coli* and purified by Ni-NTA affinity chromatography. An initial assay to determine activity was performed by adding purified enzyme to spread-plated *M. luteus* and examining plates for clear areas after overnight incubation.

Results: Overexpression produces a 20kD protein that reacts with pentaHis antibodies, as determined by SDS-PAGE and Western Blot, respectively.

Purification by affinity chromatography yields a pure, concentrated product.

Conclusions: We are currently determining the range of conditions that permit enzyme activity and the extent of its anti-*Salmonella* activity. Results of recent assays will be discussed.

087

Absence of antimicrobial resistance in emerging *Salmonella* Newport isolates in Argentina from 2001-2007.

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Purpose: *Salmonella* Newport has been identified as an emerging pathogen of concern over the last 15 years due to its increasing prevalence and alarming rate of multidrug resistance among isolates. The objective of this study was to determine the genetic diversity, antimicrobial resistance profiles, and distribution of virulence genes of *S. Newport* isolates circulating among diseased individuals in Argentina during the period of 2001-2007 and to compare these isolates to those of emerging strains in the United States during the same time period. Given the global distribution of multidrug resistant *S. Newport*, we hypothesized that the strains from Argentina would also exhibit a similar multidrug resistance profile, potentially including the plasmid-borne third generation cephalosporin resistance gene *cmx-2*.

Methods: We analyzed 126 *Salmonella* Newport strains that had been isolated from diseased individuals in Argentina during the years 2001-2007. All isolates were fingerprinted by PFGE using the enzymes XbaI and BlnI. The presence of the virulence genes *avrA*, *pefA*, and *sefD* was assessed by PCR. The susceptibility of each isolate to a panel of 13 antibiotics was tested using disk diffusion methods.

Results: All 126 isolates had the same virulence gene pattern: *avrA*(+), *pefA*(-), *sefD*(+). 116 of the 126 isolates were identified as pan-susceptible to the 13 antibiotics tested. Of the 10 isolates demonstrating resistance, eight isolates were resistant to one or two antibiotics and two were multidrug resistant. PFGE fingerprinting identified 62 different patterns, 40 of which were unique and 22 of which fell into clusters with 1-14 other identical isolates.

Conclusions: The isolates in this study were genetically diverse with almost complete absence of resistance against the antibiotics tested. The resistance trends among these samples over the course of the 7 years tested do not parallel those observed among emerging US isolates. Given the global distribution of multidrug resistant *S. Newport* and the extensive distribution of the multidrug resistance plasmid among various *Salmonella* serotypes, the lack of resistance among *S. Newport* from Argentina warrants further investigation.

088

Development of a multiplex PCR for the detection of major serotypes of Shiga-toxin producing *E. coli* in bovine feces.

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Purpose: Shiga-toxin producing *E. coli* (STEC), particularly O157:H7, are major food borne pathogens. Recently, non-O157 serotypes have also become a major public health concern. Unlike O157:H7, detection procedures for non-O157 have not been fully developed. Confirmation of non-O157 strains is generally based on agglutination with antisera, which is labor intensive and often nonspecific. Our objective was to develop a multiplex PCR to distinguish the seven major STEC serotypes (O45, O103, O121, O111, O145, O157 and O26) and evaluate whether the procedure could be used to screen feces before subjecting them to cultural procedures.

Methods: Published sequences for the O-specific antigen coding genes, *rfbE* (O157), and *wzx* and *wfqB* (non-O157), were analyzed to design primers to amplify serotype-specific regions. The specificity of the procedure was tested with pure cultures of STEC strains (n=94). Feces spiked with different concentrations of a mixture of seven STEC strains were tested before and after 6 h enrichment in *E. coli* (EC) broth. Additionally, fecal samples (12 from

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feedlot [F] and 12 from dairy [D] cattle) were collected, enriched in EC broth and subjected to multiplex PCR and cultural procedures for detection and isolation of STEC.

Results: The assay amplified product sizes of 890 (O45), 716 (O103), 587 (O121), 451 (O111), 371 (O145), 296 (O157) and 241 (O26) bp, and specificity of amplification was validated with 94 STEC strains. All seven serotypes were specifically amplified in spiked feces with a detection limit of 8.0×10^{-2} . Eight of 12 F fecal samples (1 of O45, 6 of O103, 2 of O121, 7 of O157, and 6 of O26) and 9 of 12 D fecal samples (1 of O45, 4 of O103, 3 of O121, 9 of O157, and 7 of O26) were positive for at least one STEC, with 6 F and 7 D samples contained multiple STEC. Seven of 12 F (3 of O103, 6 of O157 and 1 of O26) and 8 of 12 D (1 of O103, 2 of O121, 8 of O157 and 3 of O26) fecal samples were positive for one or more STEC by culture method. However, one sample in each group was PCR positive but culture negative.

Conclusion: The multiplex PCR is a useful confirmatory test for isolates of the seven STEC, however, applicability of the method to identify fecal samples positive for STEC requires further testing.

089

A multiplex, real-time PCR assay based on *stx1*, *stx2*, and *rfbE* for the quantification of *Escherichia coli* O157 in cattle feces.

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Purpose: Cattle are asymptomatic reservoirs for *E. coli* O157, a bacterial foodborne pathogen. The organism generally colonizes the hindgut of cattle and is shed in the feces at low concentrations. A subpopulation of cattle shed *E. coli* O157 at higher concentrations (super shedders), which has been linked to hide and carcass contamination at harvest, and is an important parameter in studying the dynamics of the organism. Cultural methods for quantifying *E. coli* O157 from cattle feces are logistically cumbersome and lack high-throughput capability. The objective of this research was to develop a multiplex, real-time PCR for quantification of *E. coli* O157 in cattle feces using *stx1*, *stx2*, and *rfbE* gene targets.

Methods: The primers and probes chosen for inclusion in this assay were based on unique sequences in the *stx1*, *stx2*, and *rfbE* loci from *E. coli* O157:H7 EDL 933. Primer efficiency and assay sensitivity were evaluated with pure cultures of a single or a mixture of five *E. coli* O157 strains. Sensitivity and specificity analyses were done with DNA extracted (two methods) from cattle feces spiked with known concentrations of *E. coli* O157. We also validated the assay with feces from cattle experimentally inoculated with *E. coli* O157. Extracted DNA was subjected to real-time PCR for quantification of *stx1*, *stx2*, and *rfbE* genes and results were compared to culture-based methods.

Results: In pure culture, the minimum detection limit of the assay was 1.4×10^3 CFU/ml and 3.6×10^3 CFU/ml for the single and mixture of five *E. coli* O157 strains, respectively. In feces spiked with *E. coli* O157, the minimum detection limit ranged from 5.7×10^3 CFU/g to 3.6×10^5 CFU/g depending on the number of strains used and the DNA extraction method. For the majority (10 of 13) of samples from experimentally inoculated animals, the mean concentration based on *rfbE*, *stx1*, and *stx2* genes was higher than the concentration of *E. coli* O157 determined by plate counts.

Conclusions: We have developed a multiplex real-time PCR to quantify *E. coli* O157 in cattle feces using *stx1*, *stx2*, and *rfbE* gene targets. Although higher sensitivity of the assay is needed for routine use, the procedure may be useful in identifying super shedder animals.

090

Impact of seasonal variation in ambient temperature on the transmission dynamics of a cattle pathogen: A modeling study of *Escherichia coli* O157:H7 infection in a dairy herd.

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Purpose: To better understand the influence of seasonal variation in ambient temperature on the population dynamics of a representative pathogen and its transmission, via free-living stages in a cattle herd.

Methods: We developed and evaluated a simulation model that incorporates into the classical Susceptible-Infectious-Susceptible (SIS) model, temperature effects on population dynamics and transmission of *Escherichia coli* O157:H7 in a dairy herd.

Results: Model results suggest that seasonal variation in ambient temperature has considerable impact on pathogen population densities on barn surfaces and in water troughs. Contaminated drinking water emerged as the most important pathway of *E. coli* O157:H7 transmission. Sensitivity analysis indicated that water-mediated transmission is amplified in the warmer seasons when the amount of drinking water available to the cattle herd is increased. Specifically, the increased prevalence of *E. coli* O157:H7 in the herd during summer is a consequence of faster replication of the pathogen favored by elevated temperature and slower turn-over rate of water leading to greater pathogen load in drinking water.

Conclusions: The model predicts that spread of infection via indirect transmission can be effectively controlled by improved drinking-water management. This includes reducing the total amount of available drinking water per animal or other strategies to reduce growth of the pathogen in water, including lowering drinking-water temperature during the warmer seasons.

091

Effects of controlled intervention strategies on the quantities of a ceftiofur resistance gene (*bla*_{CMY-2}) in the feces of feedlot cattle.

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Purpose: To investigate the effects of two intervention strategies (i.e., feeding of preventive doses of chlortetracycline following ceftiofur (Excede®) treatment and mixing of ceftiofur-treated with untreated animals at a ratio of 1:10) on ceftiofur resistance in bacteria as determined by quantifying the *bla*_{CMY-2} gene in fecal community DNA.

Methods: A controlled field trial was conducted on 176 steers (interim data presented here are from the first 88 steers). Steers were randomly allocated to 16 pens of 11 steers each. All steers in 8 pens were given ceftiofur on day 0; 4 of these pens received three 5-day regimens (with a one day break in between) of chlortetracycline (CTC) in their feed starting at day 4. In the remaining 8 pens, ceftiofur was given to only 1 steer among the 11 pen mates. Among these 8 pens, CTC was likewise given to all animals in just 4 pens. Fecal samples were collected every other day to 26 days. Community DNA from 200mg feces was extracted via the Qiagen Stool Kit in the QIAcube robot. The *bla*_{CMY-2} gene copies/μl of community DNA were determined using quantitative real time PCR. Total DNA concentration was assayed using Nanodrop®. The relationship between the quantity of *bla*_{CMY-2} gene (ln of the ratio to [total DNA]) and explanatory variables (CTC and mixing (MIX) in a full factorial design interacting with period (DAY)) was assessed using multi-level mixed model adjusting for dependence of responses within pen and within repeats by animals.

Results: A full factorial model including all main effects, 2-way and 3-way interaction terms was highly significant ($P < 0.00001$). CTC had a strong overall

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effect of increasing the gene copies consistently across other factors ($P < 0.00001$). Mixing had a period-specific effect of decreasing the gene copies inconsistently across other factors.

Conclusions: The preliminary data shows that CTC apparently favors rapid expansion of the target gene. Mixing has a significant, though inconsistent, sparing effect on the target gene. Results (*bla*_{CMV-2} gene copies) from the full dataset ($n=176$ steers), normalized to total bacterial DNA using primers specific to enteric bacteria or else *E. coli* and total aerobic bacteria c.f.u. count, will be presented.

092

A systematic review of vaccines to reduce the shedding of *E. coli* O157 in the feces of domestic ruminants.

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Purpose: Domestic ruminants, particularly cattle, are a major reservoir for *E. coli* O157, and beef products have been a significant source of human infection. Vaccines are one type of pre-harvest intervention used to reduce *E. coli* O157 in cattle. The objective of this study was to systematically review the literature on trials assessing the effect of vaccines on the reduction of *E. coli* O157 in domestic ruminants, and meta-analyze the results.

Methods: A systematic search of 10 databases was conducted to identify relevant studies published between 1990 and 2010. References were screened, and data was extracted from relevant papers. All included studies were described, and a meta-analysis was performed.

Results: The search identified 20 relevant papers, from 2004 to 2010, which detailed 24 trials involving 46 comparisons between vaccine and control groups. Nine trials involved deliberate challenges and 15 natural exposures; the 3 main vaccine types were Type III secreted protein vaccines (11 trials), SRP (4), and bacterin (3). In the natural exposure trials reporting results for fecal outcomes, vaccines significantly reduced fecal prevalence in 3 of 4 SRP and 9 of 15 Type III vaccine comparisons. Eight comparisons were included in the Type III vaccine random effects meta-analysis; the vaccine significantly reduced fecal shedding in comparison to control/placebo treatment (OR=0.38 95% CI=0.29, 0.51). There was significant heterogeneity, but no evidence of publication bias. The random effects meta-analysis of SRP vaccines involved 4 comparisons, and there was a positive effect of the vaccine (OR=0.35 95% CI=0.20, 0.61). There was no significant heterogeneity, but significant publication bias was present. However, caution should be taken in interpreting the results of the meta-analysis due to the low sample size.

Conclusions: In summary, 24 trials of *E. coli* O157 vaccines in ruminants have now been detailed in the published literature. Meta-analysis of trials for SRP and Type III vaccines suggest that the vaccines statistically significantly reduce the prevalence of *E. coli* O157 in cattle feces.

093

Occurrence of Methicillin Resistant *Staphylococcus aureus* (MRSA) on-farm, at slaughter and retail pork in commercial swine and implications for food safety.

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Purpose: The objective of this study was to determine the occurrence of methicillin resistant *Staphylococcus aureus* (MRSA) in finishing pigs. Secondly, we assess the likelihood of carriage or cross contamination at slaughter and determine the potential food safety implications.

Methods: A serial cross sectional study targeting five cohorts of commercial swine farms was conducted to determine the carriage of MRSA in the nasal cavity and peri-anal skin in market-age pigs on-farm and at slaughter, carcass swabs at slaughter and retail pork samples. The study also compares the phenotypic and genotypic relatedness of MRSA isolated on-farm, at slaughter and from retail pork. Paired nasal and peri-anal swab samples ($n=24$ /farm) were collected from market age pigs on-farm and the same batch of pigs were followed and sampled at the lairage before slaughter and carcass swabs at post evisceration stage before chilling from a total of five farms. Pork samples from the same batch of pigs were collected at retail market.

Staphylococcus aureus isolates were recovered from nasal, peri-anal and carcass swabs and retail pork samples following conventional cultural methods using oxacillin resistance screening agar. Biochemically screened isolates were tested for oxacillin resistance and for the presence of species-specific gene (*nuc*) and methicillin resistance marker gene (*mecA*) genes.

Results: The prevalence of MRSA per farm ranged from 0 to 33%. In one of the farms, MRSA isolates were recovered from same batch of pigs before slaughter at the lairage ($n=9$), carcass swabs ($n=3$) and retail meat ($n=2$). Of the total oxacillin resistant staphylococci isolates recovered from nasal ($n=74$) and peri-anal ($n=22$) swabs, 28 (37.8%) and 15 (68.2%) had *mecA* gene, respectively. The proportion of MRSA isolates detected from carcass swabs and retail pork was 10 of 13 and 5 of the 8 isolates respectively. Genotyping to determine persistence of carriage from farm to carcass is currently underway.

Conclusions: Preliminary results show MRSA is prevalent in market age pigs on-farm and at slaughter and could persist to retail pork and suggests the possible implications for food safety.

094

Prevalence and genotypic characterization of Staphylococcal isolates from Northeastern Brazil.

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Purpose: Despite the common consumption of raw milk and economic significance in the semi-arid region of Brazil, there is no information on occurrence of public health important pathogens. The objective of the study is to determine the epidemiologic relatedness of *Staphylococcus* isolates originated from milk and environmental samples using phenotypic and genotypic approaches.

Methods: The following samples were collected from 21 farms (one sample per farm): milk, water, hand swab from the milker, and sponges from the collecting bucket (from the cow), the bulk bucket (from the collecting bucket), the gate in the pen and the rope used to hobble cows. Each sample was cultured for *Staphylococcus* and biochemical tests for confirmation was done by catalase, coagulase, Vogues-Proskauer, and latex agglutination tests. Antimicrobial susceptibility testing was done by Kirby-Bauer disk diffusion. The presence of *mecA* was verified by PCR and SCCmec typing was also performed. Clonal relatedness was determined by pulsed field gel electrophoresis (PFGE) and multi locus sequence typing (MLST).

Results: *S. aureus* was isolated from 38.1% of the milk samples, 14.3% of the hand swabs, 4.8% bulk, 4.8% gate and 9.5% rope. None of the water or collecting buckets were positive. Prevalence of coagulase-negative staphylococci was 42.9% of the milk samples, 14.3% of the water, 52.4% on the hand swabs, 38.1% for samples collected from the collecting bucket and rope, 9.5% bulk, 33.3% gate, and 38.1% rope. *S. aureus* ($n=39$) and coagulase-negative staphylococci ($n=70$) exhibited antimicrobial resistance at various proportions as shown respectively: oxacillin (0, 24), penicillin (21, 21), streptomycin (14, 7) and tetracycline (8, 12). Of the coagulase-negative staphylococci, 44.2% were resistant to at least one antimicrobial.

Conclusions: Staphylococci including *S. aureus* were very common. While no MRSA was detected, the common occurrence of antimicrobial resistance

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among *S. aureus* and methicillin resistance among CoNS could be of public health concern. Further genotypic analysis by SCCmec Typing, PFGE and MLST are currently underway.

095

Antimicrobial drug resistance among enterococci from broilers fed antimicrobials and exposed poultry abattoir workers: a veterinary public health perspective.

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Purpose: We investigated the prevalence and association of antimicrobial drug resistance among *Enterococcus* spp. from the caecae of healthy broilers and abattoir workers.

Method: Broiler caecae ($n = 240$) from 6 farms were aseptically collected and transported on ice to the laboratory. Caecal content and faeces from 29 abattoir workers and 28 human controls were selectively cultured for enterococci. Using the CLSI micro-dilution broth method, MICs were determined for doxycycline, trimethoprim, sulphamethoxazole, ampicillin, enrofloxacin, fosfomycin, erythromycin, ceftriaxone, virginiamycin, vancomycin and bacitracin.

Results: Higher levels of resistance were observed among broiler isolates for most antimicrobials compared to isolates from both abattoir workers and the control group. Nevertheless, isolates from both abattoir workers and the control group also generally carried fairly high levels of resistance and no significant differences were observed between the two groups. However, the MIC₉₀ (for enrofloxacin and bacitracin) and MIC₅₀ (for doxycycline) figures suggested that abattoir workers carried levels of resistance that were similar to levels observed among broiler isolates but higher than in the control group, for antimicrobials that are extensively used in the poultry industry for therapeutic and growth enhancement purposes.

Conclusion: The findings of this study are suggestive of an association between the resistance levels of isolates from abattoir workers and broilers. As in previous studies, these results suggest that indiscriminate use of antimicrobials as feed additives in poultry is fuelling development of resistance among enteric isolates from broilers in South Africa.

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Reporting of methodological features in observational studies of pre-harvest food safety.

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Purpose: Observational studies are used in the pre-harvest food safety literature to estimate prevalence, identify risk factors, and evaluate potential mitigation strategies. Transparency in reporting the methods and results of a study are essential to allow the reader to interpret the results. Although guidelines for reporting of observational studies are available in the human healthcare field, there are no structured reporting guidelines for observational study designs in livestock species. Our objective was to evaluate the reporting of observational studies in the pre-harvest food safety literature.

Methods: One hundred studies published between 1999 and 2009 were evaluated using a structured checklist.

Results: A number of the criteria evaluated were reported in over 75% of the studies, including geographic location, definitions and sources of outcome data, organizational level and source of data for independent variables, description of statistical methods, number of herds enrolled in the study and included in the analysis, and sources of funding. Other features were not consistently reported, including eligibility criteria for groups (such as barn, room, or pen) and individuals, numbers of groups and individuals included in various stages of the study, identification of primary outcomes, the distinction between risk factors and confounding variables, the identification of a primary exposure variable, the referent level for categorical variable associations, methods of controlling confounding and missing variables, model fit, details of subset analysis, demographic information at the sampling unit level, and generalizability of the study results.

Conclusions: Improvement in reporting of observational studies of pre-harvest food safety will aid research readers and reviewers in interpreting and evaluating the results of such studies.

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Using functional metagenomics to predict the emergence of antibiotic resistance.

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Purpose: The vast majority of bacteria are not culturable by standard techniques, and consequently, the only way to discover novel resistance genes that might be located in uncultivated bacteria is to use culture-independent methods. In functional metagenomics DNA is extracted directly from a sample, and novel genes that confer a specific function of interest on a host bacterium can be investigated. The present study aimed to discover novel florfenicol resistance genes using previously-constructed metagenomic libraries. We hypothesized that genes that confer resistance to florfenicol exist in the environment and that this approach of identifying novel resistance genes, particularly those that might be found in non-cultivable microbes, can help to predict the emergence of resistance.

Methods: Metagenomic libraries were incubated at 37° C and at room temperature on LB plates with florfenicol. Clones growing on these selective plates were evaluated by restriction endonuclease analysis. Mutants exhibiting susceptibility to florfenicol following transposon mutagenesis were sequenced using the manufacturer's primers to identify the inactivated gene.

Results: A single clone from the soil of a remote site in Alaska possessed a 42-kb insert that mediated reduced susceptibility to florfenicol at 30° C but not at 37° C in an *Escherichia coli* host. The gene responsible for the reduced susceptibility was designated pexA and encodes a 415-amino acid protein, which also conferred reduced susceptibility to chloramphenicol. The PexA protein showed a structure similar to that of efflux pumps of the major facilitator superfamily but revealed little homology to known efflux pumps conferring phenicol resistance.

Conclusions: This study is an example of the ability of functional metagenomics to identify novel resistance genes in environmental bacteria. This could be a powerful tool for the approval process of new antimicrobial compounds. Databases of existing metagenomic libraries could be constructed and screened to evaluate whether a resistance mechanism already exists, and if so, to predict the rate of dissemination based on the genes to which the novel resistance genes are physically linked.

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Genotypic and phenotypic evidence for L-fucose utilization by *Campylobacter jejuni*.
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Purpose: *Campylobacter* remains among the leading causes of bacterial food-borne illness. The current understanding of *Campylobacter* physiology suggests that it is asaccharolytic and is unable to catabolize exogenous carbohydrates. Contrary to this paradigm, we provide evidence for L-fucose utilization by *C. jejuni*.

Methods and results: The fucose phenotype, shown by growth in chemically defined medium, is strain specific and linked to an 11 ORF plasticity region of the chromosome. By constructing a mutation in a putative fucose permease (*fucP*), one of genes in the plasticity region, it was found that *fucP* is necessary but not sufficient for fucose utilization, suggesting that multiple genes in the locus are required for fucose metabolism. Measured by quantitative RT-PCR, transcription of the plasticity region genes is highly inducible by fucose. PCR screening of *C. jejuni* isolates revealed a broad distribution of this genetic locus in strains derived from various host species. Birds inoculated with the *fucP* mutant strain alone were colonized at a level comparable to the wild-type strain; however in low dose (10^5 CFU per bird) co-colonization experiments, the mutant was significantly outcompeted by the wild-type strain. This advantage was not observed when birds were inoculated at a higher inoculum dose (10^8 CFU per bird).

Conclusion: An inducible locus of fucose utilization supports the *in vitro* growth of *C. jejuni*. Although not essential, fucose utilization may be advantageous during chicken colonization.

099

Salicylate functions as efflux pump inducer and promotes the emergence of fluoroquinolone-resistant mutants in *Campylobacter*
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Purpose: Salicylate, a non-steroidal anti-inflammatory compound, has been shown to increase the resistance of *Campylobacter* to antimicrobials. The aim of this study is to identify the molecular mechanism underlying salicylate-induced resistance.

Methods: A transcriptional fusion assay was used to determine if salicylate induces the expression of *cmeABC*, which encodes a multidrug efflux pump contributing to the resistance to structurally divergent antibiotics. The expression level of CmeABC was also determined by quantitative real time PCR and immunoblotting in the presence or absence of salicylate. The frequency of emergence of fluoroquinolone-resistant *Campylobacter* mutants with or without salicylate was measured using MH agar plates containing ciprofloxacin.

Results: The β -galactosidase assay revealed a dose-dependent induction of *cmeABC* transcription by salicylate. The result was further confirmed by quantitative real time PCR and immunoblotting. Salicylate did not affect the expression of CmeR, the repressor for *cmeABC*. Thus the induction of *cmeABC* is likely due to the interaction of salicylate with CmeR at the protein level. The induction of CmeABC increases the resistance to multiple antibiotics. Notably, presence of salicylate in *Campylobacter* cultures resulted in approximately 10-fold increase in the frequency of emergence of fluoroquinolone-resistant mutants.

Conclusions: Salicylate induces the production of the CmeABC efflux pump in *Campylobacter*. This induction increase *Campylobacter* resistance to antimicrobials and promotes the emergence of spontaneous fluoroquinolone-resistant *Campylobacter*.

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Molecular characterization of vancomycin-resistant and vancomycin-susceptible *Enterococcus faecium* isolated from a semi-closed and integrated agri-food system.

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Purpose: Vancomycin-resistant enterococci (VRE) have emerged as important nosocomial human pathogens in the United States. Horizontal transmission of vancomycin resistance among enterococci is largely attributed to the genetic determinant *vanA*. We earlier reported for the first time non-clinical human VRE isolated from community wastewater in a semi-closed agri-food system in Texas. The purpose of the present study was to characterize the virulence genes in enterococci that are often associated with *vanA* type resistance, perform multilocus sequence typing for across-host comparisons, and to determine the transferability of the *vanA* gene to host-specific or non-host-specific susceptible enterococcal isolates via conjugation and comparison these traits with vancomycin-susceptible *E. faecium* (VSE) isolates.

Methods: The study population consisted of 18 geographically separated locations, most with both farm and non-farm facilities, and representing both human (swine-worker and non swine-worker) and swine group-level cohorts. A total of 1,252 enterococci isolated from human wastewater samples and an equivalent number of swine fecal samples were tested for the vancomycin resistance; and when identified, the presence of *vanA* and *vanB* genes.

Results: A total of 63 human isolates were positive for the *vanA* gene and one isolate was positive for *vanB* gene. None of the swine isolates were positive for *vanA* or *vanB* genes. All *vanA* positive isolates also carried the *erm(B)* and *tet(M)* genes. Out of 63 VRE isolates, 57 were positive for *esp* (enterococcal surface protein) gene, a potent virulence factor among VRE clones. All the VRE isolates were negative for *asa1*, *gelE*, *cylA*, and *hyl* genes. The conjugation studies by both broth and filter mating revealed ready transfer of *vanA* gene to susceptible strains.

Conclusions: The potential link between the *vanA* and other antibiotic resistance determinants coupled with *esp* gene may exert pressure for biofilm formation by VRE. Further studies are being undertaken to study the differences in the clonal lineages and diversity of Tn1546 among VRE and VSE strains to better understand the potential for dissemination of VRE in agri-food production systems.

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Survey of bovine enteric viruses and water quality in the midwestern pasture streams during 2007 to 2009 grazing seasons.

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Purpose: Enterovirus is a biological index for the fecal contamination in the water and can be used to differentiate the origin of fecal contaminant because of its host specificity. Using bovine enterovirus (BEV) as target, the occurrence of bovine fecal contamination in streams passing pasture areas, which get together at Rathburn Lake in Southern Iowa, was studied during 2007-2009 grazing seasons.

Methods: A total of 13 pastures with different grazing patterns were employed for the study. Water samples (n=1,274) were collected biweekly from each stream at upper and down ends in each pasture and, after appropriate processing, tested by a multiplex real-time RT-PCR for BEV, bovine coronavirus (BCoV) and bovine rotavirus (BRV). The samples were also evaluated for total coliform bacteria count as the standard of water quality.

Results: BEV, BCoV and BRV were detected in 3.9%, 1.1% and 0.5% of the samples, respectively, suggesting that grazing cattle can be a source of fecal loading of water sources. There was no difference in incidence rate of bovine enteric viruses between farms. However, BEV incidence rate was different between up and down streams and was reduced after raining, implying dilution effect and/or loading of contaminant from the pasture. Incidence rate of BEV, BCoV and coliform bacteria was higher in summer and fall, fall and spring, respectively, whereas no seasonal difference was observed with BRV. Overall, detection of BEV in the stream was attributed to the presence of cattle in the pasture. The total coliform bacteria counts, on the other hand, were not correlated with cattle stocking density, suggesting that other sources may contribute to impairing surface water quality in the pasture stream.

Conclusions: Collectively, the study results suggest that appropriate grazing or pasture management practices should be able to control fecal contamination of pasture streams. Further study is needed for tracing other source(s) of contaminant to surface water.

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Development of reverse genetics with a full-length infectious cDNA of porcine epidemic diarrhea virus
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Porcine epidemic diarrhea virus (PEDV) is a swine-specific group 1 enteric coronavirus. Despite periodic vaccination to prevent the disease, PEDV continues to emerge in Korea causing heavy economical losses to the swine industry nationwide. In order to develop effective vaccines and control strategies, it is essential to understand the fundamental biology of PEDV. Although tremendous works on coronaviruses have been reported since the SARS outbreak, PEDV research is less invested. Furthermore, molecular genetic studies are hampered by the lack of reverse genetics that is the most powerful genetic tool in modern virology. In the present study, thus, we generated a full-length PEDV cDNA clone that enables us to greatly facilitate and profoundly advance the future study of basic viral biology. Seven contiguous fragments spanning the entire 28.3-kb PEDV genome were systematically assembled into a bacterial artificial chromosome (BAC) system as a single clone and placed under the eukaryotic CMV promoter. Upon DNA transfection of Vero cells, we tested infectivity of the constructed cDNA clone and explored its potential for the expression of a foreign gene and construction of genetically engineered recombinant viruses. Results of in vitro analysis using a reverse genetics system for PEDV will be discussed.

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Efficacy of HYPER-EGG K-99 avian egg antibodies on prevention of diarrhea and improvement of survival of colostrum-deprived neonatal calves infected with ETEC K-99 B44.

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Bovine enteric colibacillosis is an economically important disease in newborn calves caused by Enterotoxigenic *Escherichia coli* (ETEC) K-99. Fimbrial adhesins are considered to be the most important virulence factor for ETEC pathogenesis, when mucosal association plays the crucial role for intestinal colonization leading to the ETEC infection. In the present study, we investigated (i) the effect of ETEC K-99 Avian Egg Antibodies (HYPER-EGG K-99) on adhesion of ETEC K-99 to the porcine intestinal cell line, IPEC-J2 *in vitro* and (ii) efficacy of K-99 Avian Egg Antibodies for protection of colostrum-deprived neonatal calves from enteric colibacillosis. In the *in vitro* studies, ability of IPEC-J2 cell line was assessed to support adherence of ETEC K-99 bacteria, pre-incubated with K-99 avian egg antibodies or control egg powder, prepared from eggs of unvaccinated chickens. The study revealed that compare to the control egg powder, ETEC K-99-specific avian egg antibodies strongly inhibited adhesion of ETEC K-99 to IPEC-J2 cell line, in an antibody dose-dependent manner. In the *in vivo* studies, eighteen colostrum-deprived neonatal calves were challenged with 1×10^{10} - 5×10^{10} CFU of *E. coli* K-99 B44, after four hours of feeding milk replacer with either K-99 avian egg antibodies or control egg powder, prepared from eggs of unvaccinated chickens. It was demonstrated that most of the control calves 8/9 (89%) died with severe diarrhea within 48 hours post-infection. In contrast, the antibody fed calves had mild diarrhea and only 3/9 calves (33%) died by day 10 post-infection. These data suggest that avian egg antibodies specific to the ETEC K-99 fimbrial antigen can inhibit adhesion of ETEC K-99 to the intestinal cells, and the HYPER-EGG K-99 antibodies can be effective in protecting 66% of the colostrums-deprived newborn calves from enteric colibacillosis.

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Inverse relationship between fluid accumulation and adherence of enterotoxigenic *Escherichia coli* in ligated jejunal loops.

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Several studies have provided evidence that heat-labile enterotoxin (LT) produced by enterotoxigenic *Escherichia coli* (ETEC) increases ETEC adherence to the small intestinal epithelium and enhances colonization. No relationship has been detected between heat-stable enterotoxin-b (STb) production and colonization; however, an induction of net fluid secretion by either toxin might result in a reduction of bacterial adherence. The effect of expression of LT and STb by F4ac⁺ ETEC was studied in ligated jejunal assays in weaned purebred Yorkshire pigs originating from two different farms, each group conducted as an independent experiment (Exp. 1, n = 5, 8-week-old; Exp. 2, n = 6, 6-8-week-old). In both experiments, pigs tested positive for intestinal mucin-type glycoprotein (a putative F4ac receptor); in Exp. 2, all pigs tested positive for a polymorphism in the mucin 4 gene that is correlated with F4ac susceptibility. At the time of the experiment, each pig was inoculated with six O8:NM:F4ac strains, three loops per strain, as follows. (1) NaI⁸ EAST1⁺, LT⁺, STb⁺ WAM2317 parent; (2) STb⁻ (Δ estB) mutant (MUN297); (3) MUN297 complemented with STb (MUN298); (4) LT⁻ STb⁻ (Δ eltAB Δ estB) mutant (MUN300); (5) MUN300 complemented with LT (MUN301); (6) 1836-2 (non-enterotoxigenic, wild-type). *In vitro*, as detected by STb-ELISA and GM1-ELISA, STb, but not LT, were secreted into the culture supernatant by the strains that produced the respective toxins. In both experiments, only those strains that produced STb induced marked fluid accumulation in the loops, with the mean fluid accumulation in response to WAM2317 and MUN298 significantly greater than that to the other strains ($P < 0.0001$). Bacterial adherence scores based on immunohistochemistry and CFU/g of washed mucosa were both lowest in the strains that expressed STb and highest in those that did not. For data from the two experiments combined, the Pearson correlation coefficient (R) between fluid volume (ml/cm) and log CFU/g was -0.57021 ($P < 0.0001$); $R^2 = 0.3521$ (n=197). The results of this study support the hypothesis that enterotoxin-induced fluid accumulation flushes progeny organisms into the lumen of the bowel.

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Unraveling the mysteries of O-antigens in *Escherichia coli*.

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O-antigens on the surface of *E. coli* are important virulence factors that are targets of both the innate and adaptive immune system and play a major role in pathogenicity. O-antigens that are responsible for antigenic specificity of the strain, determine the O-serogroup. The designation of O-serogroups is important for classifying *E. coli* strains, for epidemiological studies, in tracing the source of the outbreaks of gastrointestinal or other illness, and for linking the source to the infection. O-serotyping is performed by agglutination reaction against antisera developed for each of the O-serogroups which is time consuming and not always accurate. We are developing molecular O-typing scheme for *E. coli*. Genes for the synthesis of O- antigen are found in the O-antigen gene cluster, which is flanked upstream by 39-bp JUMPstart sequence and downstream by *gnd* gene. DNA sequences of O-antigen gene clusters exhibit variability in different O-groups and we have developed PCR assays for many O-groups based on unique genes *wzx* and *wzy*. We are currently sequencing 102 O-antigen clusters at J. Crag Venter Institute. Comparative genomics of the O-antigen clusters and significance of genetic aberrations in the clusters will be discussed. Genome sequencing of 112 reference strains used for O-serotyping is also being pursued. A better understanding of the genetic loci encoding all O-antigens that confer pathogenicity would lead to a more comprehensive view of the structural patterns

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and biosynthetic pathways used to build such antigens and their role in pathogenesis. The genomic sequences will enable the researcher to: (a) determine the role and relevance of genes encoding the O-antigen cluster that may be implicated as determinants of pathogenicity and disease specificity in humans and animals; (b) evaluate the molecular mechanisms for host specificity associated with different O-groups in human and animal infections; (c) elucidate the molecular mechanism of host adaptation and immune system evasion; (d) identify specific genes and proteins suitable for use in the development of the next generation of diagnostic, therapeutic, and immunoprophylactic agents.

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Contribution of PhoA(cj) to Twin Arginine Translocation mediated *Campylobacter jejuni* function and resilience to Environmental Stresses. M. Drozd, D. Gangaiah, Z. Liu, G. Rajashekara, The Ohio State University, Wooster, OH, Email: drozd.6@osu.edu.

Purpose: PhoA(cj) is the only alkaline phosphatase in *Campylobacter* species; it is necessary for the uptake of phosphate from organic sources, and is transported from the cytoplasm via the Twin Arginine Translocation (TAT) system. The tatC deletion mutant presents a diverse phenotypes affecting *C. jejuni* survival under various stresses and chicken colonization. Poly P is linked to stress tolerance, formation of VBNC's, and survival defects in chicken. Here, we propose that some of the survival defects seen in the tatC deletion mutant is caused by defects in poly P regulation due to the inhibition of PhoA(cj) translocation by the TAT system.

Methods: We determined Poly P concentration, assayed for cell invasion and chicken colonization, as well as phenotypic survival under different stresses to elucidate the role of PhoA(cj) in both poly P regulation and stress responses.

Results: We found that phoA deletion causes defects in Ppk activity, upregulation of biofilm formation, as well as increased antibiotic resistance. Similar to the *V. cholera* phoX mutant, biofilm phenotypes were rescued with high levels of inorganic phosphate. PhoA(cj) is more phylogenetically, as well as phenotypically, similar to the PhoX enzymes found in common pathogenic organisms such as *V. cholera* and *Pseudomonadaceae*, than the common model organisms, *E. coli* and *B. subtilis*.

Conclusions: Our results suggest that *Campylobacter* may have additional mechanisms that allow for resilience in intestinal colonization that are not found associated with *V. cholera* PhoX. It also suggests that Poly P regulation by PhoA in *Campylobacter* is distinct from the well studied *E. coli* model, and these differences should be considered to better fit empirical phenotypes.

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Epidemiological implications of the invasion associated marker in *Campylobacter jejuni* isolated from cattle

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Campylobacter is a predominant bacterial foodborne pathogen that causes gastrointestinal illness in the US. The main species infecting humans is *Campylobacter jejuni* which commonly colonizes food-producing animals, including cattle. Attachment and invasion of *C. jejuni* to the host's gastrointestinal tract are important virulence mechanisms. However, adherence and invasion capability of *C. jejuni* strains varies, which could be attributed to the presence or absence of certain genetic determinants. Of particular interest is the invasion associated marker (*iam*) which has been associated with invasive *Campylobacter*. Therefore, the objective of this study was to investigate the role of the *iam* in chicken colonization- and human cell invasion potential of *C. jejuni* isolated from bovine. A total of 60 *C. jejuni* were isolated from fecal samples (n = 944) collected from cattle at processing plants across 4 geographical regions within the US. In addition, 13 *C. jejuni* isolates were acquired from different sporadic human infections. All *C. jejuni* were isolated following standard enrichment and selection methods and further confirmed by multiplex PCR. Random Amplification of Polymorphic DNA (RAPD) analysis showed that 24% of the bovine isolates harbored the *iam* as compared to 31% of the human isolates. Furthermore, *iam*-specific PCR showed that only 9% of the bovine isolates were *iam*-positive as compared to 54% of the human isolates. Sequence analysis of the *iam* fragment indicated that the *iam* sequences from the bovine isolates were more heterogeneous as compared to the human isolates. *iam*-positive bovine isolates that clustered with human isolates, colonized chicken cecum and invaded human INT-407 cells more efficiently, which suggested that the *iam* was polymorphic and that only certain alleles might contribute to *C. jejuni*'s colonization and invasion. *iam*-positive bovine isolates were also highly resistant to multiple antibiotics including ciprofloxacin, erythromycin, and gentamicin. These findings suggest that multi-antibiotic resistant bovine *C. jejuni* strains that are capable of colonizing multiple hosts may exacerbate the *C. jejuni* infections cycle.

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An alternative protocol for cultivation of *Lawsonia intracellularis*.

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Purpose: The aim of this study was to describe an alternative protocol for cultivation of *L. intracellularis* in cell monolayers providing necessary growth conditions without Tri-gas incubators.

Methods: The isolate PHE/MN1-00 was previously grown in murine fibroblast-like McCoy cells and stored at -72°C until use. The infected cells were incubated in two different conditions. For the conventional protocol, the cells were placed in the Tri-gas incubator with 83.2% nitrogen, 8.8% carbon dioxide and 8% oxygen and a temperature of 37°C. Flasks were removed and flushed with hydrogen daily. For the alternative protocol, the flasks were placed in a plastic bag (Original Space Bag®) which was then hermetically closed, inflated with a mixture of gas containing 10% hydrogen, 10% carbon dioxide, and 80% nitrogen, incubated at 37°C for eight days. The gas inside the bag was replaced every 24 hours and CO₂ and O₂ levels were measured at the same time. The CyQuant® cell proliferation assay was used to monitor the growth rate of infected and non-infected cells in both incubation protocols. *L. intracellularis* growth and infection monitoring were performed by immunocytochemistry (ICC) and quantitative PCR.

Results: In the alternative protocol, the daily CO₂ and O₂ levels were between 7.0-8.0% and 5.5-6.5%, respectively. The gas levels were constant in the Tri-gas incubator. Non-infected and infected cells had similar growth rates in both incubation protocols. There was no significant difference in the numbers of heavily infected cells, counted by ICC, between the two protocols. Furthermore, quantitative PCR showed similar growth curves reaching a peak concentration (10⁶ *L. intracellularis*/ml) seven days after infection.

Conclusions: Based on these results, we believe this approach can be used for static cultivation of bacteria without requiring a Tri-gas incubator. The flexibility of this protocol allows testing of various environmental conditions for *L. intracellularis* cultivation and development of diagnostic techniques. Additionally, this affordable technology gives an opportunity to engage more research institutes in this area.

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Multilocus sequencing typing system for *Lawsonia intracellularis*.

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Purpose: *L. intracellularis* is an obligate intracellular bacterium and is the causative agent of proliferative enteropathy in pigs, horses, and a variety of other species. Isolation and cultivation of this organism is extremely difficult due to its intracellular nature. For this reason very few isolates exist in pure culture making traditional strain typing techniques difficult. Previous analyses of *L. intracellularis* isolates using variable number tandem repeat (VNTR) sequencing, demonstrated a clear distinction between the isolates obtained from porcine and non-porcine species. The objective of this study was to develop a multilocus sequence typing (MLST) system for *L. intracellularis* based on genetic variation in seven housekeeping genes to determine genetic relationships among isolates and confirm the strain differences found with VNTR sequencing.

Methods: The MLST system was established using six *L. intracellularis* pure culture isolates of porcine, equine, and rodent origin. Primers were designed for the seven loci and specificity was ensured by PCR with other enteric bacteria and closely related species.

Results: The allelic profiles or sequence types, generated from these sequences showed that *L. intracellularis* is genetically diverse. While the porcine isolates had identical sequence types, the equine and rodent isolates were distinctly different from both the porcine isolates and from each other.

Conclusions: Application of this sequencing approach to further field isolate collections may create a universally accepted nomenclature and a more global picture of the epidemiology of this organism.

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A rabbit infection model for equine proliferative enteropathy.

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Purpose: To demonstrate the susceptibility of rabbits to *Lawsonia intracellularis* (LI) from a clinical case of equine proliferative enteropathy (EPE), and thus provide an animal model for investigations on LI pathogenesis and therapy in horses.

Methods: Twelve juvenile does were randomly assigned to 4 groups. Three groups (Gr. 1, 2, and 3) were orally inoculated with LI differing in concentration (per rabbit) and passage in cell culture [p.#] as follows: Gr. 1) 1.3×10^8 LI [p.9]; Gr. 2) 2.5×10^8 LI [p.10]; and Gr. 3) 1.6×10^7 LI [p.7/8]. Gr. 4) media only. Rabbits were monitored daily for clinical signs and weight changes. Feces and blood were collected prior to infection and at 7, 14 and 21 days post infection (d PI) and stored at -20°C until analysis. For 3 weeks PI, 1 doe per group was humanely euthanized and multiple intestinal samples were collected. Tissues were stained by immunohistochemistry (IHC) with a LI-specific mouse monoclonal antibody. Amount of label was graded semi-quantitatively from 0 to 5. Serum IgG titers were measured using an immuno peroxidase monolayer assay. Fecal samples were analyzed by a quantitative PCR for LI fecal shedding.

Results: No significant difference ($p=0.6$) was found in bodyweight changes among groups. No serologic response was detected until 14 d PI, when Gr. 1 and 2 developed high titers (480 and 1920 respectively). By 21 d PI, the same groups had >1920 titers. Gr. 3 had titers of only 30 at 14 d PI. No serologic response was detected in Gr. 4 (controls). Fecal shedding of LI was present only in Gr. 2 on 7 d PI; it was detected in Gr. 1 and 2 on 14 d PI. No shedding was noted on 21 d PI in any group or at any timepoint in Gr. 3 or 4. Gross lesions of congestion, thickening and a "cobblestone" appearance of jejunal and ileal serosae were apparent in Gr. 1 and 2 on 14 d and 21 d PI, being milder on the latter time point. IHC confirmed LI antigen in the enterocytes of Gr. 1 and 2 on 7, 14 and 21 d PI. No lesions or IHC staining were found in Gr. 3 or 4.

Conclusions: This animal model simulates natural EPE, as typical LI lesions were produced using a cell cultured equine isolate of LI in juvenile rabbits, with minimal adverse health effects. Dose of challenge appeared to impact the degree of disease observed.

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Functional cloning of novel antibiotic resistance genes in chicken gut microflora.

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Purpose: A recent report (Sommer et al., 2009. *Science*. 325:1128), using a culture-independent approach, showed immense diversity of antibiotic resistance (AR) genes in the human gut microbiome. We hypothesize that food animal gut microflora also contain diverse and novel AR genes which could contribute to future emergence of AR resistance in pathogens important in animal and human health.

Methods: Here we examined AR reservoir in chicken gut microflora using a metagenomic-based functional cloning method. The total genomic DNA was extracted from cecal contents of two individual free range chickens. The DNAs were physically sheered into 1 to 3 kb fragments, cloned into vector pZE21-MCS, and transformed into *E.coli* TOP10 strain, resulting two metagenomic libraries of a total size of 10^9 base pairs per library. The positive clones from the libraries were selected by plating on plates containing one of the 12 antibiotics of interest. The inserts conferring AR resistance were sequenced and the AR genes were annotated and aligned with the homologs deposited in the public database GenBank.

Results: Three AR genes were identified to confer resistance to beta-lactams (Ampicillin, Penicillin, Amoxicillin) or aminoglycoside (Spectinomycin, Streptomycin). Two of the three genes showed low sequence similarity to the closely related gene in GenBank (as low as 38% at amino acid level), indicating that they are evolutionally distant from known resistance genes.

Conclusions: Together, the findings from this study demonstrated the effectiveness of the metagenomic approach for examination of AR reservoir in food animals, and revealed novel AR resistance genes in chicken gut microflora.

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Bacterial profiling of enteric microbiota of reticulated giraffes (*Giraffa camelopardis reticulata*).

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Purpose: The objective of this study was to characterize the normal enteric microbiota of six healthy giraffes in a zoological collection. Changes in the composition of the enteric microbiota of herbivores can result in severe gastrointestinal disease and death which can be catastrophic in zoological collections where large herbivores such as elephants, rhinoceros and giraffes are very valuable, and likely difficult to replace. Understanding the normal composition of enteric bacterial populations is essential for further investigating the role they play in health and disease.

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Methods: Fecal samples were collected from the enclosures of six giraffes and stored on ice then frozen until processing. The outside layer of each pellet was removed and genomic DNA extracted from the remaining feces using a commercially available kit. Polymerase chain reaction of the 16S rRNA gene was performed followed by denaturing gradient gel electrophoresis. Bacterial profiles of fecal pellets were compared within the same animal, to determine differences in distribution of bacteria within different pellets from one excretion. Bacterial profiles from different animals were compared to each other.

Results: Results showed almost identical DGGE patterns between three fecal pellets of the same animal, suggesting an even distribution of bacterial colonies between pellets. Distinct profile patterns on DGGE analysis, however, were noted between all six giraffes despite being on identical diets. Two mother baby pairs also showed distinct patterns between mother and offspring.

Conclusions: This study represents the first characterization of the normal enteric microbiota of captive giraffes. It also provides valuable information regarding methodology for collecting fecal samples of giraffes for diagnostic testing.

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Survival of *Mycobacterium avium* subsp. *paratuberculosis* in bovine monocyte-derived macrophages.

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Purpose: Johne's disease is a significant problem in many North American cattle herds. The efficacy of currently available vaccines is questionable. There is a need to develop efficacious vaccines and strains of *Mycobacterium avium* subsp. *paratuberculosis* (MAPTB) that could serve as potential candidates for live attenuated vaccines. The confirmation of attenuation requires reliable methods to assess reductions in virulence. In these studies, we characterized the ability of wild-type and transposon insertion mutants of MAPTB to invade and survive in bovine monocyte-derived macrophages. Methods: Blood was obtained from feedlot animals and mononuclear cells were separated by density-gradient centrifugation. Adherent cells were retained in 24-well tissue culture plates with RPMI medium. Macrophage cultures were inoculated with either wild-type MAPTB (K-10) or transposon insertion mutant strain 4H2 (derived from K-10) at an MOI of 10. Cell culture supernatant medium or cell lysates were diluted and cultured on Middlebrook agar, with mycobactin supplement to enumerate surviving bacteria at 2, 24 and 72 hours. Results: There was little difference in surviving bacteria at 2 hours, but the numbers of surviving 4H2 bacteria were reduced by approximately 15% and 45% at 24 and 72 hours, respectively. A second assay was developed using the monocyte-derived macrophages in 48-well plates. The adherent macrophages were inoculated with descending MOI ratios of 10^2 through 10^{-4} in replicates of three or four wells. After a 4-hour inoculation, the macrophage cultures were incubated for an additional 24 hours. The macrophages were lysed and plated to detect the presence of viable bacteria. The number of bacteria required to infect 50% of the macrophages (MID₅₀) was over 10-fold higher for the 4H2 strain of MAPTB. Conclusions: The results indicated that the 4H2 strain was not phagocytosed and/or did not survive as well as the K-10 strain in macrophages. Continued development of macrophage attenuation assays (e.g., the MID₅₀ method) may be necessary to compare possible vaccine candidates. Final confirmation of attenuation must be achieved in immunologically competent host animals.

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Effect of adjuvants on recombinant coccidia antigen vaccination.

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Purpose: A study was conducted to investigate the effects of Montanide™ adjuvants in emulsion with a recombinant protein in a vaccination trial against avian coccidiosis. Body weight gains, fecal oocyst shedding, and humoral and intestinal cytokine responses were evaluated before and after oral challenge

Methods: Chickens were immunized by subcutaneous injection with a purified *Eimeria* recombinant protein, either alone or mixed with Montanide™ ISA 70 VG or ISA 71 VG.

Results: Immunization with Montanide™ ISA 70 VG or ISA 71 VG significantly ($P < 0.05$) increased body weight gains compared to vaccination with antigen alone. Recombinant protein formulated with Montanide ISA 71 VG also reduced fecal oocyst shedding compared to vaccine formulated without adjuvant. All vaccine with adjuvants enhanced serum antibody titers. Increased levels of gene transcripts encoding IL-2 and IFN- γ were observed while levels of IL-10 and IL-15 mRNAs were reduced in the intraepithelial lymphocytes from duodenum. Finally, increased infiltration of CD8⁺ and TCR $\gamma\delta$ ⁺ lymphocytes at the site of immunization was observed in animals injected with Montanide ISA 71 VG based vaccine.

Conclusions: These results suggest that vaccination with the *E. acervulina* recombinant protein in combination with Montanide™ adjuvants triggers protective immunity against avian coccidiosis.

Key words: Coccidiosis; Vaccine; Adjuvant, Chickens

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Lecithinase production by *Clostridium perfringens* HBS and LDA isolates utilizing drop-plate method of enumeration in conjunction with microplate and agar egg yolk assays.

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Purpose: Hemorrhagic bowel syndrome (HBS) is an emerging, highly fatal disease of adult dairy cattle. It is associated with heavy enteric growth of *C. perfringens* type A. The primary virulence factor of this bacterium is alpha toxin, a highly lethal exotoxin that destroys lecithin, a component of cellular phospholipids. The goal of this study was to compare lecithinase activity from *C. perfringens* type A isolates obtained from the jejunum of dairy cattle with HBS to that of herd mates with left displaced abomasum (LDA).

Methods: Isolates were grown in sodium thioglycollate and growth curves were established by collecting spectrophotometric data every 15 minutes. A portion of each culture was harvested at 3 time points along the mid to late log phase of growth curves, and from 1 time point 3 hours into the stationary phase of growth. To minimize oxygen exposure and eliminate any potential bias that could result from a disparity in oxygen tolerance between isolates, a drop plate method was utilized to determine colony forming units (CFU) per ml: 10ul drops from eight dilutions of each harvested sample were plated in quadruplicate on blood agar. The drop plating method allowed the time efficient determination of CFU/ml for several isolates from multiple dilutions and time points. Colony counts were performed with the aid of digital photography and imaging software. Clarified supernatants from the 4 time points were analyzed for lecithinase content by microplate and egg yolk agar assays. After incubation for 24 hours, lecithinase units were calculated by comparison to

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standard curves made on each microplate and each agar plate.

Results: Preliminary results indicate *C. perfringens* isolates from the HBS group appear to produce more lecithinase than do isolates from the LDA group as measured at the late log/early stationary phase of growth.

Conclusions: Evidence of the role of the ubiquitous organism in the pathogenesis of HBS can serve as a foundation for further research into genomic characteristics and source of organism, trigger events for toxin production in the bowel, and targeted mitigation strategies for this disease.

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Hypervirulent *Clostridium difficile* in pigs its public health significance.

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Purpose: The objectives of the project are to investigate whether pigs carry hypervirulent strains of *C. difficile* and to compare phenotypic and genotypic attributes of strains of human and porcine origin.

Methods: Fecal samples (n=251) were collected from swine farms in Ohio (n=3 farms) and North Carolina [NC] (n= 5 farms) at farrowing, nursery, and finishing. Bacteriology was done using conventional approaches. Antimicrobial susceptibility was tested using Epsilometric test for ciprofloxacin, erthromycin, metronidazole, vancomycin, tetracycline, and ampicillin.

Results: Prevalence of 74.5% within farrowing piglets, 0.45% within nursery pigs, and 0% within finishing pigs were found. Within farrowing pigs, the prevalence in NC was 65.6% and in Ohio 88.5%. A low level of multi-drug resistance was found, although most samples, 81.5% (106/130), had a ciprofloxacin MIC >32 (highly resistant). Genotypically, the majority of isolates, 82.6% (428/518), were toxin A+B+. On a pig level, 82.5% (161/195) were found to carry *C. difficile* stains which are Toxinotype V, binary toxin positive and have a 39 bp deletion in the *tcdC* toxin down regulator gene. Twenty-four pigs (12.3%) were also found to carry more than one strain of *C. difficile*. Characterization of human isolates (n=24) found two strain types similar to those found in swine: Toxinotype O and nontoxogenic. PFGE findings show a high level of genotypic diversity among isolates of swine origin, with clustering among farms. These results also show a group of Toxinotype V strains of swine origin with 100% similarity to CDC NAP7 isolate of human origin.

Conclusions: While the absence of *C. difficile* at finishing stage of production is encouraging, the occurrence of hypervirulent strains in swine may have significant implications to public health.

Conclusions: While the absence of *C. difficile* at finishing stage of production is encouraging, the occurrence of hypervirulent strains in swine indicates the public health significance of *C. difficile* of porcine origin.

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Regulation of antigen specific T-cell responses by paracrine vitamin D signaling in peripheral blood mononuclear cell cultures.

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Purpose: The active metabolite of vitamin D₃, 1,25-dihydroxyvitamin D₃, is synthesized from 25-hydroxyvitamin D₃ by the enzyme 1 α -hydroxylase. Toll-like receptor signaling has been shown to induce 1 α -hydroxylase gene expression in monocytes. Consequently, endogenous synthesis of 1,25-dihydroxyvitamin D₃ from 25-hydroxyvitamin D₃ in monocytes regulates innate immune responses of monocytes. In this study we wanted to determine if 1 α -hydroxylase was expressed in antigen-stimulated PBMCs and if antigen-specific immune responses were regulated by endogenous synthesis of 1,25-dihydroxyvitamin D₃ from 25-hydroxyvitamin D₃ in PBMC cultures.

Methods: PBMCs were isolated from *Mycobacterium bovis* (*M. bovis*) BCG-vaccinated bull calves (n = 7) and cultured for 24 hours with 0 or 10 μ g/ml purified protein derivative of *M. bovis* (*M. bovis* PPD) and 0 or 100 ng/ml 25-hydroxyvitamin D₃. Gene expression in the PBMCs was then measured using real-time PCR. Cultured PBMCs also were sorted based on markers for monocytes (CD14), B-cells (IgM) and T-cells (CD3) using FACS. Gene expression was then measured in the sorted cells.

Results: *M. bovis* PPD induced 1 α -hydroxylase gene expression in PBMC cultures. Furthermore, addition of 25-hydroxyvitamin D₃ down-regulated IFN- γ and IL-17F gene expression in stimulated PBMC cultures. Sorting the stimulated PBMCs revealed that 1 α -hydroxylase expression was induced in the monocytes and B-cells, but not in the T-cells, and that addition of 25-hydroxyvitamin D₃ to PBMCs down-regulated antigen-specific IFN- γ and IL-17F responses in the T-cells.

Conclusions: Evidence from this study indicates that activated monocytes and B-cells synthesize 1,25-dihydroxyvitamin D₃ and that 1,25-dihydroxyvitamin D₃ from the monocytes and B-cells down-regulates antigen-specific expression of IFN- γ and IL-17F in T-cells in a paracrine fashion. Therefore, 25-hydroxyvitamin D₃ availability, which depends on dietary intake of vitamin D and sun exposure, is an important aspect in regulating adaptive immunity.

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Preliminary investigation of B cell immune responses to rotavirus in vitamin A deficient piglets.

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Rotaviruses (RV) are the major cause of viral gastroenteritis in infants and young animals. Currently available human RV vaccines are effective in the developed countries but have reduced efficacy in Africa and Asia, where RV diarrhea is an important cause of childhood mortality. Micronutrient deficiencies including low Vitamin A may result in poor health and reduced RV vaccine efficacy in such children. Piglets resemble infants in gut physiology, anatomy, nutritional requirements and immune responses, and unlike mice, they are susceptible to human RV diarrhea. Our objective was to establish a Vitamin A deficient (VAD) piglet model to study the effect of Vitamin A and its adjuvancy on immune responses to RV vaccines. VAD piglets (n=13) and normal piglets (n=9) derived from sows fed VAD or conventional diets, were orally vaccinated (attenuated human RV) and/or supplemented with vitamin A and/or challenged with virulent human RV. Serum and hepatic vitamin A levels were measured by HPLC. RV specific antibody (Ab) responses and Ab secreting cells (ASCs) were assessed by ELISA and ELISPOT, respectively. Based on a limited number of piglets per group to date, the following trends were noted. Vitamin A levels in deficient piglets were lower both in serum (at 1 day of age) and liver (on necropsy) compared to normal piglets. VAD piglets that were vaccinated or vaccinated and supplemented with vitamin A had lower serum and intestinal IgA Ab responses than normal piglets, which coincided with the longer duration of RV-induced diarrhea and shedding in VAD challenged piglets. Conversely, normal piglets had lower serum IgG Ab titers and reduced numbers of gut IgG ASCs compared to VAD piglets, suggesting reduced systemic exposure to RV. The frequency of B cells in the blood (pre and post-challenge) and duodenum (post-challenge) of VAD piglets were lower than those of normal piglets. In summary, we established a VAD piglet model for RV vaccine studies. Preliminary findings suggest that VAD piglets have impaired B cell immune responses to RV as compared to normal piglets, confirming the hypothesis that reduced levels of Vitamin A in children from developing countries may result in reduced efficacy of oral RV vaccines.

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Analysis of Bovine Viral Diarrhea Viruses-infected monocytes: Identification of cytopathic and non-cytopathic biotype differences.

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Purpose: Pathogenesis of the disease caused by BVDV is complex, as each BVDV strain has two biotypes: non-cytopathic (ncp) and cytopathic (cp). Using proteomics, we evaluated the effect of cp and ncp BVDV infection of bovine monocytes to determine their role in viral immune suppression and uncontrolled inflammation.

Methods: Proteins were isolated by differential detergent fractionation and identified by 2D-LC ESI MS/MS.

Results: We identified 137 and 228 significantly altered bovine proteins due to ncp and cp BVDV infection, respectively. Functional analysis of these proteins using the Gene Ontology (GO) showed multiple under- and over-represented GO functions in molecular function, biological process and cellular component between the two BVDV biotypes. Analysis of the top immunological pathways affected by BVDV infection revealed that pathways representing macropinocytosis, virus entry via endocytic pathway, integrin signalling and primary immunodeficiency signalling were identified only in ncp BVDV-infected monocytes. In contrast, pathways like actin cytoskeleton signalling, RhoA signalling, clathrin-mediated endocytosis signalling and interferon signalling were identified only in cp BVDV-infected cells. Of the six common pathways involved in BVDV infection, acute phase response signalling was the most significant. In addition, we observed a major involvement of mitochondria transport-related proteins in oxidative phosphorylation and apoptosis pathways in BVDV-infected monocytes that could explain the biological effect of BVDV biotypes on the survival or death of immune cells. This and other findings by proteome analysis are supported by Western blotting and Flow Cytometry.

Conclusion: In conclusion, the combined use of multiple approaches facilitates a better understanding of host-pathogen interactions by revealing significant functional differences in the host proteins that respond to cp or ncp BVDV infection.

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Bovine macrophages cleave extracellular traps produced by macrophages and neutrophils in response to Mannheimia Haemolytica and its leukotoxin. N. Aulik, K. Hellenbrand, C. Czuprynski, University of Wisconsin Madison, Madison, WI, Email: naaulik@wisc.edu.

Human neutrophils actively release nuclear DNA studded with antimicrobial compounds to form neutrophil extracellular traps (NETs) that ensnare and kill pathogens. Previous studies demonstrated that DNase I in human serum degrades NETs. Although macrophages do not possess DNase I, they do produce DNase II that is required for the breakdown of apoptotic cell corpses and ejected erythroblast nuclei. Here, we show bovine macrophages cleave neutrophil and macrophage extracellular traps. Western blot analysis demonstrated the presence of DNase II. Co-incubation of NETs with bovine macrophages resulted in loss of NETs. Adding DNase II inhibitors diminished extracellular trap degradation by bovine macrophages. Mouse and human macrophages displayed a similar ability to eliminate extracellular traps. These data indicate a possible role for macrophages in the remodeling and removal of extracellular traps.

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Histophilus somnii causes Neutrophil Extracellular Trap (NET) formation in bovine neutrophils.

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Histophilus somnii causes respiratory, reproductive, cardiac and neurological diseases in cattle. It has been reported that bovine neutrophils are limited in their capacity to phagocytose and kill *H. somnii*. Recently, it was discovered that neutrophils can produce neutrophil extracellular traps (NETs) in response to pathogens. In this study, we found that NET formation occurs in response to *H. somnii* cells as quantified by release of extracellular DNA in the absence of LDH release. We observed that NETs formed in response to *H. somnii* can trap and kill a portion of the extracellular bacterial cells. We examined the ultrastructure of bovine NETs produced in response to *H. somnii* using scanning electron and confocal microscopy, and observed bacterial cells trapped within NETs. Evidence suggests that vesicles produced by *H. somnii* contribute to NET formation. We hypothesize that NETs play a role in the host response to *H. somnii* infection in cattle.

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Up-regulation of inflammatory mediators and pro-apoptotic genes during Mycoplasma gallisepticum infection.

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Purpose: The avian pathogen, Mycoplasma gallisepticum, is known to cause severe immunopathology in the tracheal mucosa of chickens by initiating infiltration by heterophils, lymphocytes, and macrophages. However, the molecular events which induce the recruitment of inflammatory cells to the respiratory mucosa have not been well characterized.

Methods: To evaluate the pathologic role of certain inflammatory mediators such as chemokines, cytokines, Toll like receptor's, and cytolytic molecules, we used Affymetrix microarray technology and quantitative RT-PCR to determine the relative expression levels of representative genes during experimental infection (compared to PBS controls). In situ hybridization (ISH) was conducted using riboprobes to target representatives of these gene classes (CCL-19, IL-10, and IFN- γ) in order to understand the cellular context of gene expression. TUNEL assay was utilized to assess cells undergoing apoptosis in tracheal tissue.

Results: Chemotactic factors up-regulated in the tracheal mucosa of infected birds compared to control birds were CCL-19, CXCL-13, lymphotactin and IL-6. Higher expression levels of chemokines such as RANTES, MIP-1 β , CXCL14 and MIP-2 were also observed. ISH revealed that CCL-19 is predominately expressed by follicular lymphocytes. Surprisingly, both IFN- γ and IL-10 mRNA levels were up regulated, with no significant changes in the levels of IL-1 β , TNF- α , or IL-6. Both IFN- γ and IL-10 were expressed by lymphocytes in tracheal tissues. Higher expression levels of TLR 1, 2 and 4 were also observed. Cytolytic molecules such as granzyme A, NK lysine, and certain pro-apoptotic genes, such as fas, caspase 3 and 8, were also up-regulated during infection. Large numbers of apoptotic cells were detected in the tracheae of infected birds through TUNEL assay, consistent with the up-regulation of pro-apoptotic genes.

Conclusions: Several chemokines, regulatory cytokines, TLR's and various cytolytic and apoptotic proteins are important mediators in the inflammatory response to Mycoplasma gallisepticum in chickens. Additionally, lymphocytes were found to be a key effector cell in this response.

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Use of dermal fibroblasts to evaluate developmental, and between-animal variation in innate immune response.

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Characterization of endogenous variation in the innate immune system may lead to isolation of markers for selection of animals with enhanced disease resistance. As a model system, dermal fibroblasts were isolated from 15 heifers at approximately 5 and 11 months of age and used to characterize the in vitro production of IL-8 in response to stimulation with E. coli lipopolysaccharide (LPS) as well as interleukin-1 β (IL-1 β). Dermal fibroblasts were stimulated with 100 ng/mL LPS and 1 ng/mL of IL-1 β for 36 and 24 hours respectively. Response to each ligand was assayed using IL-8 ELISA specific to the bovine system. IL-8 response between heifers showed a high degree of variability (four-fold difference between top and bottom quintiles; $P < 0.01$) at each age with the ranking of low to high producer showing consistency across the two time points. IL-8 response to LPS increased ($P < 0.01$) in fibroblasts isolated from 10-12 month heifers ($1.34 \pm .53$ ng/mL) in comparison to those at 4-6 months ($.294 \pm 0.22$ ng/mL). Similarly, the IL-8 response to IL-1 β increased ($P < 0.01$) from the later biopsies (4.88 ± 2.08 ng/mL) in comparison to earlier samples (1.72 ± 0.95 ng/mL). The change in response between the two biopsies could signal an important developmental aspect in the bovine innate immune response. The relation of cellular to whole animal responses was then determined through an in vivo LPS-challenge conducted on heifers having the lowest ($n=4$) and highest ($n=4$) responding fibroblasts. Animals were administered LPS (0.5 ug/kg BW) via jugular catheter then blood samples and body temperatures were obtained at hourly intervals. Unexpectedly, animals with low responding fibroblasts showed significantly higher ($P < 0.01$) temperature increases than animals with high responding fibroblasts. These results document substantial variation in innate immune signaling in cultured fibroblasts that appears related to an animal's systemic response to LPS. The fibroblast model may be of interest in evaluating genetic basis of between-animal variation in innate immunity.

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Response of cultured fibroblasts to LPS reflects in vivo response to *E. coli* mastitis.

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Innate immunity has major role in resisting bovine mastitis. Variation in innate immunity may cause differential outcomes in different phases of mammary gland infection. We have explored between-cow variation in the ability of their dermal fibroblasts to respond to inflammatory stimuli (IL-1B or *E. coli* LPS) by producing IL-8 as a marker of the innate immune response. Subsequently, we assessed the effect of the variable response on different phases of experimental *E. coli* mastitis. Skin biopsies obtained from 43 mid-lactation cows were used to establish dermal fibroblast cultures. Confluent cultures were challenged with LPS (100 ng/ml) and separately with IL-1B (10 ng/ml). After 24 h the media concentrations of IL-8 in response to LPS was determined and used to stratify the animals. Groups (n=8) of the lowest and highest responding cultures were deemed to have come from low and high responder animals, respectively. Average IL-8 measured in response to LPS was much greater ($P<0.01$) in the high (1155 ± 30.96 pg/ml) than low (318.9 ± 32.78 pg/ml) responders (n=8). The IL-1B-induced IL-8 concentrations in the high responder fibroblasts (9331 ± 676.6 pg/ml) were approximately 4-fold greater ($P<0.01$) than with the low responder cells. The in vivo response was then evaluated during late-lactation in 4 high- and 4 low-responder cows from which the fibroblasts had been collected. Cows were challenged with *E. coli* (strain P4; 200 cfu) in one quarter. All cows developed clinical mastitis in the challenged quarter. All cows cleared the infection within 5 days, but milk BSA concentration, an indicator of tissue damage, was higher ($P<0.05$) in high responders compared to that of low responders. Milk SCC began to decline later in the high responder group and as a result was significantly higher ($P<0.05$) than the low responder group from 4 to 10 days post-infusion. These data suggest that the high innate response phenotype resulted in a higher level of inflammation and a longer delay in SCC reduction, two key indicators of milk quality.

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Distribution of polysulfated proteoglycans in the equine digital lamellae: implications for the pathogenesis of laminitis.

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Purpose: Equine laminitis is a crippling and frequently fatal condition in which the connective tissue surrounding the distal phalanx separates from the epithelia which synthesizes the hoof wall leading to rotation and sinking of the distal phalanx within the hoof capsule. Recent work from our laboratory has demonstrated the upregulation of the gene ADAMTS4 in the developing stages of laminitis - the earliest marker of disease development. ADAMTS4 is known to cleave aggrecan and versican and has been implicated in the development of osteoarthritis. To date, however, the presence of these core proteins, as well as their associated polysulfated glycosaminoglycans, have not been shown in the equine digital lamellae.

Methods: Aggrecan, versican, hyaluronan, keratan sulfate, and chondroitin sulfate were localized using indirect immunofluorescence within 10µm sections of flash frozen, unfixed lamellar tissue collected from healthy animals and those with Obel Grade III laminitis.

Results: Aggrecan, versican, hyaluronan, keratan sulfate, and chondroitin sulfate were all shown to localize to the secondary epidermal lamellae of healthy horses, and most likely create a protective, hydrated extracellular matrix (ECM) and possibly intracellular matrix around and within the basal epithelial cell layer. In addition, chondroitin sulfate was shown to be highly enriched in the secondary dermal lamellae, possibly associated with proteoglycans that interact with tensile collagen fibers. In tissue collected from animals with Obel Grade III stage lameness, the secondary epidermal lamellae shows a highly significant decrease in versican consistent with a global change in epithelial cell function, while the secondary dermal lamellae contains large areas of tissue devoid of ECM.

Conclusions: Loss of versican from the epidermal ECM may subject basal epithelial cells to damaging compression forces, while disruption of the lamellar dermal ECM, presumably by ADAMTS-4 independent processes, may compromise the dermal lamellae and basement membrane junction, thus jointly contributing to separation of the dermal and epidermal lamellae in laminitic tissue.

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Depression of gene expression and elevated ADAMTS-4 degradation of versican in equine laminitis

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Equine laminitis is a devastating disease affecting the structural integrity of the digital lamellae, the tissue that suspends the horse's axial skeleton within the hoof. Loss of lamellar function in laminitis is thought to result from degradation of lamellar basement membrane components and neighboring extracellular matrix. We used cDNA-specific probes and RT-qPCR to detect the expression levels of genes encoding large sulfated proteoglycans, aggrecan and versican in normal and laminitic lamellae. The gene encoding versican was found to be significantly depressed in the late stage of experimentally induced laminitis, while gene expression of ADAMTS-4, which is a secreted MP with a disintegrin domain and thrombospondin motif, is hugely elevated in the early stage. The protein products of these genes were detected by Western blotting after SDS-PAGE of lamellar extracts. Versican neoepitope generated by ADAMTS-4 cleavage was significantly increased accompanied by elevated ADAMTS-4 enzyme activity. Large polysulfated proteoglycans are important structural components of cartilage conveying resistance to compression forces hence their cleavage by ADAMTS-4 may contribute to the failure of digital lamellae integrity in equine laminitis.

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Environment affects interferon-gamma (IFN-γ) production in neonatal foals through possible effects on dendritic cells.

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While IFN-γ plays an important role in protection against viral and intracellular bacterial infections, its production in neonates is deficient. Over time, IFN-γ production increases in the foal, eventually reaching adult levels. The mechanism responsible for this increased expression is unknown, though it is presumed that exposure to environmental antigens plays a role. We hypothesize that exposure of foals to higher concentrations of air-borne microbial antigens promotes IFN-γ expression in their lungs. We also propose that this exposure affects the function and/or maturation of dendritic cells (DCs) in the lung. Five neonatal pony foals were placed in separate stalls in a barn three times a week, for 4 hrs at a time until they were 2 months old. A second group of age-matched foals were maintained on pasture throughout the study. Air samples were collected from the barn and pasture using an impact air sampler, MAS-100eco. The bacteria and fungi collected on the petri dishes were counted and identified. Bronchoalveolar lavage (BAL) samples were collected from both groups of foals at 2, 4, and 8 weeks of age for analysis. Surface staining of DCs in the BAL was analyzed by flow cytometry. Expression

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of cytokine and cell surface antigen mRNA in BAL samples were analyzed by RT-PCR. There were significantly more bacteria and fungi in air samples collected from the barn compared to the pasture. This corresponded to significantly higher frequency of IFN- γ ⁺ cells and increased mRNA expression of CD83, TNF- α , IL-6, IL-12 in the BAL cells from those foals exposed to the barn air. However, there was no significant difference in mRNA expression of CD3 (T cells), CD80 (macrophages), CD11a (neutrophils), or CD66 (granulocytes) in BAL cells between the two groups. The frequency of DCs and the percentage of CD86⁺ DCs in BAL were also higher in foals exposed to the barn environment. In conclusion, the air in the barn contains a higher load of microbial antigens which promoted localization of DCs to the lung, without promoting an influx of inflammatory cells. This could lead to enhanced IFN- γ production through cytokine or co-stimulatory molecule expression by these activated DCs.

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Interplay of antimicrobial peptides and interferons in Porcine Reproductive and Respiratory Syndrome Virus infections.

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Antimicrobial peptides and type I and type III interferons are heterogeneous peptide families that are central to innate and adaptive immune responses. Although antiviral activities of interferons are well known, antimicrobial peptides are also recognized and increasingly characterized for antiviral activity. The porcine arterivirus, porcine reproductive and respiratory syndrome virus (PRRSV), is a devastating pathogen for the swine industry and continues to be an immunological challenge. Because early studies indicated that many antimicrobial peptides had antiviral activity, we reasoned that some porcine antimicrobial peptides may be antiviral effectors during PRRSV infection. In addition to differential expression patterns of porcine antimicrobial peptides in lungs of PRRSV-infected pigs, direct inactivation studies indicated that some porcine defensins and cathelicidins may be promising candidates for limiting PRRSV infectivity.

Similar to antimicrobial peptides, type I interferons are a group of innate immune effectors prominent in antiviral responses. However, in most species, only two subtypes, interferon-alpha and interferon-beta, have been well studied. Based on current understanding of the porcine genome, we have conducted a near complete analysis of porcine type I and type III interferons. This analysis indicated that porcine type I and type III interferons have diverse expression profiles and antiviral activities against PRRSV. Collectively, these studies identify important properties of antimicrobial peptides and interferons related to the control of PRRSV infection that should be considered in anti-PRRSV therapies and vaccines.

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Mucosal immunization with biodegradable PLG- nanoparticles elicits effective anti-PRRSV immune responses in Pigs

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Porcine reproductive and respiratory syndrome (PRRS) is an economically important endemic viral disease of swine. Currently used PRRSV vaccines administered intramuscularly impart partial or no protection against re-infections and heterologous PRRSV. Due to the inherent advantages of mucosal vaccines to effectively control viral diseases, there is an urgent need to develop a protective PRRSV mucosal vaccine. In this study the role of nanometric microspheres in delivery of PRRSV antigens to pigs was assessed. Killed PRRSV (VR2332) antigens were entrapped in poly (DL-lactide-co-glycolide) microspheres with protein encapsulation efficiency around 50%. Encapsulated microspheres were 50-400 nm in size as determined by the scanning electron microscopy. Internalization of microspheres entrapped PRRSV antigens by alveolar macrophages and dendritic cells (DCs) was quantified by flow cytometry. Confocal microscopy was performed to study localization of internalized microspheres entrapped PRRSV antigens by alveolar macrophages. Our preliminary data suggest that microspheres were able to deliver the encapsulated PRRSV antigens to early endosomes at both 3 and 12 hrs post-treatment. Intranasal immunization of pigs with microspheres entrapped PRRSV antigen revealed an increase in the frequency of myeloid cells in BAL, TBLN and Blood at PID-30. In particular, DCs were increased in blood (3-fold) and lungs (1-fold) and both immature and mature alveolar macrophages were increased 6-7 fold. In addition, there was a 2-fold increase in the frequency of $\gamma\delta$ T cells. Higher levels of IFN- α and IL-6 were detected in serum of microsphere inoculated pigs as compared to control pigs. Upon *in vitro* re-stimulation with killed PRRSV antigen, IL-6 was secreted at higher levels by lung immune cells and immunosuppressive cytokine IL-10 was reduced in blood and lungs of microsphere inoculated pigs. These results suggest that biodegradable and biocompatible microspheres represent an ideal adjuvant system with potentially widespread application in the induction of both circulating and mucosal immunity against PRRSV. This ongoing project is supported by National Pork Board and OARDC to RJG.

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Replication competent recombinant PRRS viruses expressing indicator proteins and antiviral cytokines.

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Porcine reproductive and respiratory syndrome virus (PRRSV) can subvert early innate immunity, which leads to ineffective adaptive immunity. Elucidating and overcoming immune subversion are critical for the development of effective vaccines and other control measures. We have constructed a series of recombinant PRRS viruses using an infectious PRRSV cDNA clone. Coding regions of exogenous genes, which include *Renilla* luciferase (Rluc), green and red fluorescent proteins (GFP and DsRed, respectively) and several interferons (IFN), were inserted into the infectious clone. The Rluc, GFP and DsRed constructs were replication competent in MARC-145 cells and porcine macrophages. In contrast, the replication of IFN-expressing viruses (as well as co-infected wild type viruses) was attenuated, which correlated with the anti-PRRSV activity of exogenous recombinant interferons. Because constructs expressing IFN subtypes that inhibit virus replication might be poor candidates for a modified live virus (MLV) vaccine, we are working to optimize an engineered virus that expresses an IFN subtype that does not substantially inhibit replication of vector PRRSV, but has immunomodulating activity in enhancing B- and T-cell responses. In summary, engineered PRRS viruses expressing multiple indicator proteins allow high-throughput elucidation of the role of host factors in PRRSV infection. Furthermore, some replication competent IFN-expressing viruses may be candidates for modified live virus vaccines, which could ameliorate the subverted innate immune response and potentially induce enhanced adaptive immunity against PRRSV infection.

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Induction of robust influenza A-specific IFN-gamma responses in pigs using alphavirus-derived replicon particles.

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Purpose: Two studies were conducted to evaluate the induction of influenza A-specific IFN-gamma responses in pigs using alphavirus-derived replicon particles (RP) expressing influenza A nucleoprotein (NP) and hemagglutinin (HA).

Methods: ELISpot and flow cytometry were used to measure antigen-specific IFN-gamma response and to indicate the presence of cell mediated immunity in both young pigs and gilts. Both novel H1N1 and H3N2 whole virus were used as the stimulating antigens in the ELISpot and flow cytometry assays. The first study consisted of two groups of thirty pigs each. Group 1 received RP vaccine expressing NP and Group 2 received a control RP vaccine expressing PRRSV ORF 6. The second study consisted of three groups of six first parity gilts. Group 1 received RP vaccine expressing H3 HA. Group 2 received a control RP vaccine expressing PRRSV ORFs 5 and 6. Group 3 had no treatment and was a strict negative control.

Results: In the first study Group 1 demonstrated a significant increase in the numbers of IFN-gamma secreting cells compared to Group 2 on the day of boost as well as 21 days post boost when stimulated with both novel H1N1 and H3N2. In second study Group 1 had significantly higher numbers of CD4+ CD8+ IFN-g+ and CD4+ CD8- IFN-g+ cells than Groups 2 and 3. Group 1 also demonstrated a significant increase in CD4- CD8+ IFN-g+ cells when compared to Group 3 and trended higher than Group 2 when stimulated with H3N2 influenza A. ELISpot results indicated an elevated IFN-gamma response in Group 1 when compared to both Group 2 and Group 3, although this increase was not statistically significant ($p=0.057$) when comparing Group 1 to Group 2 and Group 1 to Group 3.

Conclusions: These results indicate that the alphavirus-derived replicon particle platform expressing influenza A NP and HA can elicit a robust influenza A-specific IFN-gamma immune response in pigs.

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The expression of potentially protective dual oxidase enzymes (Duox1 And Duox2) in the female bovine reproductive tract.

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Purpose: Mucosal immunity for sexually transmitted viral, bacterial, and parasitic diseases (STDs) in animals and man. Infertility, preterm delivery, abortion and other complications can be economically and clinically significant. Understanding the innate immune response at the mucosal surface of infection is essential to enhancing protection and controlling STDs. Recently the dual oxidase enzymes (Duox1 and Duox2) have been shown to have protective effects at the mucosal level in the respiratory and digestive tracts. Duox dependent H₂O₂ generation associated with peroxidase and thiocyanate secretion has been demonstrated at the apex of respiratory and digestive tract mucosal cells. No published studies so far have examined the mucosal surfaces of the reproductive tract, although our laboratory has recently demonstrated Duox expression in the mouse reproductive tract. The aim of this study was to determine the presence of Duox1 and Duox2 in the reproductive tract of cattle using immunohistochemistry (IHC) and molecular approaches.

Methods: Ovary, oviduct, uterine mucosa, vagina and intestine were obtained from 6 adult cows at slaughter for quantitative PCR (qPCR) analysis of Duox1 and Duox2 gene expression and immunohistochemistry to identify the specific location of expression. For normalization, 18S gene expression was used as an endogenous control.

Results: qPCR results indicated expression in all reproductive tissues, with the highest gene expression of both Duox1 and Duox2 in the vagina, and the lowest in the ovary. Statistical analysis of gene expression of each tissue versus intestine showed both Duox1 and Duox2 to be higher in the vagina relative to the intestine. For IHC, specific binding of both Duox1 and Duox2 antibodies was noted in all tissues.

Conclusion: This study has demonstrated that Duox1 and 2 are present in the reproductive tract of cattle, and are most highly expressed in the vagina, the likely portal of entry for STDs. Additional studies will focus on the response of Duox molecules to reproductive tract infection.

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Mucosal adjuvanticity of *M.tb* whole cell lysate to PRRSV live vaccine leads to a better immunostimulation and protection against PRRSV challenge.

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Porcine respiratory and reproductive syndrome (PRRS) is an economically devastating disease of swine worldwide. Current PRRSV vaccines are limited in their protective efficacy. We have recently identified *Mycobacterium tuberculosis* whole cell lysate (*M.tb* WCL) as a potent mucosal adjuvant to a PRRSV live vaccine. In this study, pigs were immunized intranasally with PRRSV-MLV and *M.tb* WCL, then challenged using homologous (vaccine strain, VR2332) or virulent heterologous PRRSV (MN184) and euthanized on days post-challenge (DPC) 15, 30 and 60. Histopathological analysis was performed on formalin fixed and H&E stained lung sections, and lesions were scored. Disruption of lung architecture area and interstitial pneumonia was more evident in unvaccinated challenged pigs as compared to the mucosally vaccinated and PRRSV challenged pigs at all DPC. In particular, at DPC-30, unvaccinated and MN184 challenged pigs had severe lung lesions. Nitric oxide (NO⁺), important in innate immune responses to various viral infections and intracellular pathogens was analysed by Griess reagent. The NO production in lungs of mucosally vaccinated MN184-challenged pigs was significantly higher at DPC 30 and 60 than in unvaccinated challenged controls. Lung mononuclear cells (LMNC) and Peripheral blood mononuclear cells (PBMC) were in vitro restimulated with killed PRRSV antigens and further, proliferation study using a nuclear dye CFSE, by flow cytometry, and proliferation index (PI) analyzed by flowjo software. The proliferation of total lymphocytes, and particularly cytotoxic T lymphocytes (CTLs) (CD8 β ⁺ cells) from the lungs of mucosally vaccinated and MN184 challenged pigs was higher than unvaccinated challenged pigs. At DPC-30, there was a 2- fold increase in the PI in PBMCs of vaccinated and MN184 challenged pigs. The lung-MNCs of vaccinated and VR2332 challenged pigs showed greater than 3-fold increase in the PI of CTLs. In conclusion, *M.tb* WCL shows better immunostimulation as evidenced by higher NO production, increased T-lymphocyte and CTL proliferation and reduced lung pathology. This project was supported by National Pork Board and OARDC to RJG.

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Efficacy of canine influenza virus (H3N8) vaccine in dogs co-infected with CIV and *Streptococcus equi zooepidemicus*.

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Purpose: Canine Influenza virus (CIV) was first reported as an epizootic of dogs in 2003 in Florida among racing greyhounds, caused by an H3N8 equine influenza virus. CIV infections of dogs have since occurred throughout the U.S., possibly due to interstate transport of racing greyhounds. CIV infections have also been reported in shelters, kennels, and dog day care centers. Outbreaks have generally been limited to the affected facilities, and most dogs remain serologically negative to H3N8. CIV signs can vary from subclinical to severe disease with mortality. As with all influenza viruses, secondary bacterial infections and/or underlying conditions contribute to disease severity. In the current study, the only licensed CIV vaccine, a product of Intervet Schering Plough Animal Health, was tested for efficacy in reducing severity of disease caused by a combination of CIV and *S. equi zooepidemicus*.

Methods: A vaccination trial was conducted in which vaccinates were compared to controls following single or dual agent challenge. Parameters included serology, clinical scores, viral and bacterial shedding, and pathology.

Results: It was found that co-infection led to more severe disease than infection with either agent alone. Importantly, CIV (H3N8) vaccine was shown to significantly reduce the severity of disease and viral shedding in both single and dual infected dogs.

Conclusions: These findings are important because most, if not all, CIV infected dogs will also be infected with a variety of bacteria commonly found in the canine respiratory tract. To achieve protection, it is important that dogs be vaccinated at least three weeks before exposure to CIV. This killed vaccine requires two doses be given not less than two weeks apart. Immunity can be expected approximately seven days after the second dose. To ensure protection, dogs at risk of CIV, such as those that are shown, kenneled frequently, attend dog day care or go to indoor training facilities regularly, should be given two doses of CIV (H3N8) vaccine two weeks apart, and then held for seven days before being placed in contact with other dogs.

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Differential expression of the porcine heavy chain immunoglobulin repertoire following Porcine Reproductive and Respiratory Syndrome Virus (PRRSV) infection.

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Effective antibody responses are critical in immunity to viral infections. In PRRSV infections, the antibody response is variable in neutralization function, antigenic specificity, and duration. Whether the variation is due to the response capacity of individual swine or other factors is not known. To address this question, we compared the heavy chain immunoglobulin mRNA diversity of healthy and PRRSV-infected pigs to determine the effect of PRRSV infection on antibody repertoire expression. Total RNA was isolated from lymphoid tissues (spleen, palatine tonsil, inguinal lymph node, and mesenteric lymph node) from two pigs experimentally infected with PRRSV JA142 at three weeks of age and sacrificed 62 days later, and two uninfected control pigs. Complementary DNA was synthesized, and PCR amplified using heavy chain-specific antibodies directed against conserved sequences within the variable region. In order to facilitate differentiation between infected and uninfected samples, two sets of primers containing unique molecular barcodes were used for amplification. The PCR products were then pooled and sequenced using Titanium 454 (Roche) pyrosequencing. Approximately 450,000 high quality reads were generated and were evenly distributed between the infected and uninfected groups. Statistically significant proportional differences were observed in the expression of variable (V) gene segments between infected and uninfected pigs. Novel low-level expression of a previously described pseudo V gene segment containing multiple stop codons was observed, as well as novel expression of joining (J) gene segments J1 and J3, thereby increasing the known complexity of the functional immunoglobulin repertoire in pigs. We expected that the results of this experiment will allow for the identification of additional, as of yet, unidentified germline V gene segments in the heavy chain locus on chromosome 7. The findings will facilitate identification of specific anti-PRRSV epitopes involved in the immune response to PRRSV and will help in the development of preventive and therapeutic strategies using protective elements of the porcine anti-PRRSV antibody repertoire.

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A time course for increased susceptibility to *Staphylococcus aureus* respiratory infection post-influenza in a swine model.

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Bacterial super-infections following influenza A virus (IAV) are predominant causes of morbidity and mortality. The emergence of methicillin-resistant *Staphylococcus aureus* (MRSA) and highly virulent IAV strains enhances the importance of understanding the mechanisms of viral-bacterial synergy. Development of an appropriate animal model to study the increased susceptibility to secondary *S. aureus* may provide important information regarding disease pathogenesis. Pigs are natural hosts to both IAV and *S. aureus* and have respiratory physiology and immune responses comparable to humans, making them an ideal model to examine polymicrobial infections. To establish a time course of susceptibility to *S. aureus* infection after IAV infection, nursery pigs infected intranasally with 10⁷ TCID₅₀ influenza A/Swine/Minnesota/1145/2007 (H3N2) for 3, 4, 5 or 6 days (D) were challenged with 10⁸ CFU of MRSA. Single pathogen control animals were utilized in this study and mock-infected pigs received 1 mL of phosphate buffered saline. Dual infected animals were harvested 48 hours following bacterial challenge. We found that lung pathology peaked in dual infected animals harvested on D6, while it decreased over time in IAV only animals. The bacterial CFU in the lung was highest in dual infected animals harvested on D5, and decreased over time. Analyses of bronchoalveolar lavage fluid with flow cytometry indicated differences between treatments. Further investigation of pro-inflammatory (IL-1 β , IL-6, TNF- α) and immunoregulatory (IL-10, IFN- γ) cytokine genes in the lungs, lymph nodes, and spleen revealed differences in the pathology of dual infected animals in comparison to sham and single infected pigs. These results demonstrate that the intranasal challenge model in nursery pigs to understand the pathogenesis of IAV and *S. aureus* co-infection would be ideal.

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Brucella outer membrane vesicles-polaxamer mixture as a vaccine for *B. melitensis* in a mouse model.

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Brucellosis is the most common zoonotic disease worldwide. There is no vaccine available for use in humans. Most brucellosis vaccines for animals are attenuated, live strains and present certain limitations. Use of bacterial outer membrane vesicles (OMVs) presents a safer alternative to induce protective immunity in the host without the risk of infection. OMVs have been successfully used as a vaccine for the prevention of meningitis caused by *Neisseria meningitidis* in humans. The aim of the present research effort was to determine the potential of Brucella OMVs as vaccine for *B. melitensis* in mice. Further, the ability of polaxamer, P85, as an adjuvant to enhance the immune response induced by OMVs was tested. Polaxamers are nonionic triblock copolymers of poly-(propylene oxide) and poly-(ethylene oxide). P85 is an amphiphilic polaxamer and has been shown to have immuno-adjuvant activity. *B. melitensis* OMVs were isolated by a combination of differential centrifugation and extensive washing. Electron microscopy showed that OMVs contained double membrane structure and ranged in size from 50-120 nm. Coomassie blue stained SDS-PAGE gels showed a range of proteins with major

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protein bands between 20 and 37kDa. Mice were vaccinated intra-muscularly and boosted two weeks after the primary vaccination. The immunized mice were challenged intraperitoneally with wild type *B. melitensis* 16M. Bacterial clearance was studied by determining the colony forming units (CFUs) in spleen and the efficacy of the vaccine was statistically analyzed. IgG1 and IgG2a antibody titers were determined in the serum of vaccinated mice. Upon in vitro stimulation of splenocytes, INF-gamma and IL-4, were determined. The potential of *Brucella* OMVs and P85 to enhance the efficacy of OMVs as a vaccine for *B. melitensis* in mice will be discussed.

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FMDV serotype O₁ peptide-based vaccines incorporating xeno-epitopes refocus humoral immune responses away from a major decoy epitope to an alternative immunogenic site.

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Purpose: Foot-and-mouth disease virus (FMDV) causes a debilitating vesicular disease of cloven-hoofed animals and is responsible for major economic losses in the livestock industry. The persistence of infection within populations, and individual hosts, is due in part to the emergence of antigenic variants within neutralizing epitopes on the viral capsid, thereby leading to the generation of escape variants. One of the most important neutralizing epitopes of most FMDV serotypes is the "G-H loop" of VP1, which spans amino acids ~130-160 and plays an important role in host cell binding and internalization. There are at least two distinct B-cell epitopes encoded within the G-H loop, and sequence comparison of 118 type O₁ strains revealed a hypervariable region at amino acid position 137-142. As no known function or secondary structure has been assigned to this region, we and others have speculated that it functions as a decoy epitope which likely contributes to the evolution of escape variants. Thus, refocusing the immune response to more highly conserved epitopes elsewhere on the viral capsid may be important in developing effective vaccines.

Methods: To address this hypothesis, cyclic peptides were synthesized with amino acids 137-142 replaced by B-cell epitopes from unrelated viruses (PRRSV or HIV), with wild-type G-H loop peptides serving as controls. Serum was collected from BALB/c mice immunized with these peptides, and the cross-reactivity of the antisera was assessed by ELISA.

Results: As anticipated, type-specific (homologous) reactivity was observed in antisera from all vaccinated animals; however, broad cross-reactivity was only observed in antisera from animals vaccinated with the PRRSV and HIV containing cyclic peptides.

Conclusions: These data support the hypothesis that immune refocusing to a B cell epitope outside of the aa137-143 region had been accomplished. Given these results, it may be possible to design chimeric viral capsid-based vaccines in which animals are not "imprinted" to generate antibodies against the hypervariable 137-142 epitope, thereby reducing the likelihood of inducing irrelevant responses. USDA grant # 58-1940-5-520.

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Epitope mapping of the E2 glycoprotein of Classical Swine Fever Virus

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Purpose: Classical Swine Fever Virus (CSFV) is a pestivirus related to Bovine Viral Diarrhea Virus and Border Disease Virus of sheep. Administration of live attenuated vaccine protects against the disease but allows for virus circulation. A marked vaccine could be useful for transformation from vaccination to a non-vaccination strategy and later as an emergency tool for limited vaccination. The E2 surface glycoprotein is the major viral immunogen that could be altered using reverse genetics techniques at antigenic level and used as a marker. The present work is focused on more detailed analysis of antigenic sites in the E2 of CSFV.

Methods: A set of 28 hybridomas was obtained producing monoclonal antibodies specific to recombinant and native E2 protein of CSFV, strain Shimen.

Results: Ten hybridomas have been obtained using native E2 protein, and eighteen - with baculovirus-produced recombinant protein. Analysis of their specificity has revealed 8 different antigenic epitopes in the E2 protein. Five new previously not described non-overlapping linear B cell epitopes have been mapped using a set of 32 overlapping synthetic peptides. Two of the newly found epitopes are located in predicted domain A, three have been found in a folded region of the E2 protein. The region of 834-856 a.a. that contains 2 linear epitopes, is structurally different between different pestiviruses. The monoclonal antibodies that have been obtained using native E2 protein, can be used in sandwich ELISA for antigen detection.

Conclusions: These findings could be used for development of marked CSFV strains and ELISA tests.

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G-CSF analogue treatment increases peripheral neutrophil numbers in pigs - a potential alternative for in-feed antibiotics.

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Immunomodulators is a promising area for therapeutic, prophylactic, and metaphylactic use to prevent and combat infectious disease. Granulocyte colony-stimulating factor (G-CSF) enhances neutrophil production and release from the bone marrow and is already licensed for use in humans for treatment of neutropenia. A limitation of cytokines as immunomodulators is their short half-life thus limiting their usefulness as a one-time injectable in production animal medicine. Here we report on a porcine G-CSF analogue created on the basis of previous findings of two point mutations in human G-CSF that resulted in increased in vitro potencies and increased ligand half-life. Our initial investigations were on the effects of G-CSF to induce and sustain a neutrophilia and leukocytosis in pigs. The G-CSF products tested included both the human product Neulasta (pegfilgrastim) as a subcutaneous injection and a mutated form of porcine G-CSF delivered via a replication-defective human adenovirus 5 vector (Ad5/G-CSF). Pigs given a single subcutaneous injection of Neulasta at 5 µg/kg, 25 µg/kg, 50 µg/kg or 100 µg/kg had a dose-dependent peak neutrophilia by 1 day post injection that ranged from an average of a 4-, 7-, 10- or 16-fold increase in neutrophil counts, respectively. Neutrophil counts following Neulasta treatment remained elevated compared to pretreatment values for 4-7 days, again in a dose-dependent manner. By comparison, pigs given one injection of the Ad5/G-CSF intramuscularly (10¹⁰ PFU) also had a neutrophilia (>2-fold increase) by 1 day post injection, however, the neutrophil counts peaked between days 3 to 11 post-treatment with a range of 6- to 8-fold increases in peak neutrophil counts. Neutrophil counts remained elevated compared to pretreatment values for up to 21 days. Examination of bone marrow response and disease efficacy studies on G-CSF treatment for the prevention of disease with common swine pathogens is ongoing. G-CSF has the potential to eliminate or reduce the need for antibiotic usage for the prevention or treatment of infectious disease, especially during typical times of stress and peak pathogen shedding and exposure such as post-weaning and post partum.

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Temporal genes expression induced by MDV in Marek's disease-resistant and -susceptible inbred chickens

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Purpose: Cancer has become the top one killer of human health worldwide in recent years, with 15-20% caused by viruses. To study the mechanism of virus-induced tumorigenesis in human and animal we use a chicken disease model, Marek's disease (MD), which is a natural generated lymphoma caused by chicken herpesvirus Marek's disease virus (MDV).

Methods: In this study we use a chicken microarray to identify the differentially expressed transcripts in the spleen samples from both infected and non-infected birds of three inbred chicken lines (line 63, line 72 and RCS-M) with different susceptibility to MD.

Results: Gene expression profiles were influenced by MDV infection in three chicken lines at different MDV progression periods. At early cytolytic phase (5 days post infection, dpi), more genes with mRNA expression differences were found in line 63 chickens which are resistant to MD; at latency stage (10dpi) more genes were found differentially mRNA expressed in RCS-M chicken which show intermediate resistance to MD; at late cytolytic phase, most differentially expressed genes are found in line 72 chickens which are susceptible to MD. Using principal component analysis (PCA), we found similar gene expression patterns between lines 63 and RCS-M as compared to line 72 both before and after MDV infection. We found that the expression of genes related to immune system activation and regulation (CTLA-4, CD8 α and CD8 β) and tumorigenesis (USP18, TNFRSF6B and MMP2) were changed by MDV infection in different chicken lines and at different time points. However the changes vary amongst the chicken lines with different susceptibility to MD. Multiple pathways were also found differentially enriched at each time point in different chicken lines.

Conclusions: Differentially expressed genes and pathways induced by MDV infection in three chicken lines are associated with different susceptibility to MD and their relationships. The functional genes and pathways in the study provide important clues for further investigation of the mechanism of etiology and viral disease resistance.

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Effect of serum composition on the functionality of bovine peripheral blood mononuclear cells.

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The periparturient period is marked by metabolic, hormonal and immunological changes which have an impact on the incidence of diseases. In a previous study, the slower transition into milking was assayed by milking cows once a day (1X) during the first week of lactation in comparison to twice a day (2X). Milking 1X was associated to lower level of metabolites such as non esterified fatty acids (NEFA) and β -hydroxy butyric acid (BHBA). Nevertheless, functions of immune cells collected on these animals were not improved when cells were cultivated in rich medium *in vitro* suggesting that the periparturient immunosuppression is more related to the metabolic milieu composition than to autonomous defect of immune cells. Therefore, we evaluated the effect of serums collected from those cows early and mid lactation of the previous study on immune cells functions. Proliferation of PBMCs was depressed by early lactation serums ($P < 0.001$) but this depression was of smaller magnitude for serums collected from cows milked 1X ($P < 0.01$). PBMCs proliferation was inversely correlated to the level of NEFA ($R = -0.86$, $P < 0.001$) and BHBA ($R = -0.54$, $P = 0.003$). Supplementing mid lactation serum with NEFA to the level observed in early lactation reduced proliferation to the level observed in early lactation. Supplementing mid lactation serum with BHBA did not affect PBMCs proliferation ($P > 0.05$). Secretion of interferon- γ was reduced in PBMCs incubated in serums collected in early lactation ($P < 0.001$), but there was no significant effect of milking frequency. Supplementation of mid lactation serums with NEFA reduced interferon- γ secretion ($P < 0.001$). In conclusion, limiting the increase in NEFA at the beginning of the lactation is essential to maintain immune functions.

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TLR dependent dendritic cell activation and clearance of *Brucella abortus* vaccine and pathogenic strains *in vivo* and *in vitro*.

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Purpose: *Brucella* are Gram-negative intracellular bacteria causing infertility in livestock and disease in humans. *B. abortus* strain 2308 is one of the pathogenic strains affecting cattle and humans. *B. abortus* rough strain RB51, lacking the O-side chain of lipopolysaccharide, is the live attenuated USDA approved vaccine used in cattle. No approved human vaccines are available. Limited information is known regarding how *Brucella* induces innate immunity. Toll like receptors (TLRs) 2, 4 and 9 are the most critical in recognizing *Brucella* by the host. However, it is not clear which TLR is most crucial in inducing DC function upon vaccination with strain RB51 or infection with strain 2308. No studies have been published addressing the role of TLRs in clearance of either smooth *B. abortus* strain 2308 or strain RB51 from intranasally infected mice.

Methods: In this study, we used strain RB51 and strain 2308 to stimulate bone marrow derived dendritic cells (BMDCs) from TLR2, 4 or 9 knockout (KO) BALB/c mice *in vitro* to differentially analyze DC activation based on upregulation of MHC class II, CD40 and CD86 surface markers. Experiments *in vivo* were also conducted to assess clearance of pathogenic strain 2308 compared to vaccine strain RB51 from intranasally infected TLR 2, or 4 or 9 KO vs. control BALB/c mice at day 14 and 42 post infection (PI). Clearance was assessed based on colony forming units (CFUs) from lung, mediastinal lymph node (MLN) and spleen.

Results: Overall, strain RB51 induced significantly ($p < 0.05$) more DC activation and function compared to strain 2308. Assessing the effect of TLRs, DCs from TLR2 KO mice induced significantly less CD86 expression than TLR4, and 9 KO and control mice. Additionally, strain RB51 induced TLR 2 and TLR9 dependent TNF- α production and TLR 2 and TLR 4 dependent IL-12 production. Assessing *in vivo* clearance, strain RB51 had a TLR4 dependent clearance at day 14 PI; and pathogenic strain 2308 had TLR2 dependent clearance at day 42 PI.

Conclusion: These results show that TLR mediated signaling is important in DC function upon strain RB51 stimulation. Additionally, there is a TLR dependent clearance *in vivo* of strain RB51 and strain 2308 in an organ dependent manner.

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Immune responses of equine respiratory epithelial cells to equine herpesvirus-1.

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Equine herpesvirus-1 (EHV-1) is the cause of respiratory disease, abortion and myelitis in horses worldwide. The first step in EHV-1 pathogenesis is infection of the upper respiratory tract epithelium. Despite the increasingly recognized importance of the early innate immune response, immunity to EHV-1 at the epithelial cell barrier remains poorly characterized. For this reason, we have recently established a primary equine respiratory epithelial cell culture (EREC) model to study immunity to EHV-1.

Four-week old differentiated ERECs were infected with EHV-1 strain Ab4 at a multiplicity of infection of 10 and cytokine mRNA responses were determined at 12, 24 and 48 hours using quantitative real-time polymerase chain reaction. In addition, Major histocompatibility complex (MHC)-I and MHC-II as well as toll-like receptor (TLR) 3 and TLR9 protein expression were examined at 24 and 48 hours using fluorescence activated cell-sorting analysis (FACS).

Infection with Ab4 resulted in increased cytokine mRNA expression (IL-1, TNF-alpha, IFN-alpha, IFN-beta, IL-6 and IL-8) and increases of TLR3 and 9 protein expression when compared to uninfected ERECs. In contrast, we found that infection with Ab4 caused marked decreases of MHC-I and MHC-II expression.

These results provide an initial characterization regarding the early immune response to EHV-1 at the epithelial cell barrier. Similarly to what has been reported for other alpha herpesviruses, there is evidence that EHV-1 modulates expression of MHC-I and MHC-II, which are important molecules for antigen presentation. In the future the EREC system will be used as a tool to further study the role of individual EHV-1 immunomodulatory genes and their mechanisms using EHV-1 deletion mutants.

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Porcine Reproductive And Respiratory Syndrome Virus (PRRSV) in serum and oral fluid samples from individual boars: Will oral fluid replace serum for PRRSV surveillance?

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Introduction: Previous experiments used pen-based oral fluids to monitor porcine reproductive and respiratory syndrome virus (PRRSV) and porcine circovirus (PCV) 2 infections in wean-to-finish populations. The purpose of the present study was to determine whether oral fluid samples could be used to monitor individually-housed adult boars for PRRSV infection.

Methods: In 3 trials, 24 boars, 5.5 months to 4 years in age, were intramuscularly (IM) inoculated with a modified-live PRRSV (MLV) vaccine (Trial 1), a Type 1 PRRSV isolate (Trial 2), or a Type 2 isolate (Trial 3). Oral fluid samples were collected daily and serum samples were collected twice weekly. Following the completion of the study, samples were randomized and blind-tested for PRRSV by real-time quantitative reverse-transcription polymerase chain reaction (qRT-PCR).

Results: With the exception of 2 boars in Replicate 2, all boars were successfully trained to provide oral fluid samples. A total of 2088 oral fluid samples were attempted, i.e., 29 days x 24 boars x 3 trials, and 1954 (93%) samples were collected. The average volume of oral fluid collected per boar across all trials was 17.9 ml (range: 1 to 39 ml). PRRSV was detected in oral fluids at DPI 1 and all oral fluid specimens were PRRSV qRT-PCR positive at DPI 4.

Although PRRSV was detected in both serum and oral fluid specimens through DPI 21, a comparison of matched samples from individual boars showed that oral fluid was equal to serum for the detection of PRRSV at DPI 7 and more likely to be positive than serum on DPI 14 and 21. Overall, oral fluid was superior to serum for the detection of PRRSV using PCR over the 21 day observation period in this study.

Conclusions: The results of this experiment provided evidence that oral fluid sampling could be an efficient, cost-effective approach to PRRSV surveillance in boar studs and other swine population.

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Detection of Anti-PRRSV antibodies in oral fluid samples from individual boars using a commercial PRRS ELISA.

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Purpose: Oral fluid samples are easily collected and their use in surveillance of PRRSV and PCV2 in group-housed pigs under both experimental and field settings is well documented. Previously, we reported shedding of PRRSV in oral fluids collected from individually penned boars over the course of 21 days post inoculation (DPI). The purpose of the present study was to determine whether PRRSV infection in individually-housed adult boars could be monitored by measuring anti-PRRSV antibodies in oral fluid samples.

Methods: In 3 trials, 24 boars, 5.5 months to 4 years in age, were intramuscularly (IM) inoculated with a modified-live PRRSV (MLV) vaccine (Trial 1), a Type 1 PRRSV isolate (Trial 2), or a Type 2 isolate (Trial 3). Oral fluid samples were collected daily and serum samples were collected twice weekly. Following the completion of the study, samples were randomized and blind-tested for anti-PRRSV antibodies by using commercial ELISA for the detection of PRRSV serum antibodies (HerdChek® PRRSV X3 ELISA, IDEXX Laboratories, Inc.).

Results: Anti-PRRSV antibodies were detected at 9 DPI and were detected throughout the sampling period.

Conclusions: A preliminary assessment of the anti-PRRSV antibody response in oral fluid suggests that this approach may be very useful for monitoring PRRSV infections in commercial swine herds.

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Development of a new immunochromatographic strip assay to detect the infection caused by PRRSV in pigs.

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A rapid immunochromatographic strip assay (ICSA) was developed for the detection of antibodies specific to nucleocapsid (N) protein of porcine reproductive and respiratory syndrome virus (PRRSV). In this study we have used a new engineered recombinant plasmid (pGEX-N) and a modified *E. coli* strain to rise the expression cassette to optimize the amino acid sequence at the GST-N junction and to introduce *E. coli* preferred codons in the recombinant GST-N sequence. Using this new engineered plasmid we succeeded in obtaining highly level expression of soluble PRRS N protein and used it in ICSA as capture antigen. The performance of this assay was evaluated with sera samples from both clinical and experimentally infected piglets.

Detection by ICSA was compared with detection by standard, available commercially; indirect enzyme-linked immunosorbent and western blot assays. The ICSA detected antibodies in sera known to contain antibodies to PRRSV in 96.74% (sensitivity) of samples from experimentally infected pigs, the specificity was 99.34% for clinical and experimental serum samples, respectively. These tests were found more simple and easy to use for a rapid and effective diagnosis of PRRS viral infection and could be used as an alternative to the ELISA for the screening of pig infected herds under field conditions.

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Influenza antibody detection in experimentally inoculated swine over time using a commercially available nucleoprotein ELISA.

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Purpose: Evaluate a commercial nucleoprotein (NP) ELISA assay for influenza antibody detection using serum samples collected over time from swine experimentally inoculated with contemporary H1N1 or H3N2 influenza virus. **Methods:** The study was performed using 82 PRRSV-, SIV- and *Mycoplasma hyopneumoniae*-negative piglets. A subgroup was vaccinated with a multivalent commercial influenza vaccine followed by a booster vaccination 21 days later. All pigs were randomly assigned to one of 3 treatment groups: negative control, Ohio '07 H1N1 inoculated, Illinois '09 H3N2 inoculated. Inoculated pigs received 2ml of virus inoculum ($10^{5.5}$ TCID₅₀/ml) intratracheally. Serum was collected on DPI -42, -21, -7, 0, +7, +14, +21, +28, +35 and +42 and stored at -20°C. At study completion, all serum samples were randomized, and assayed (same day, same technician) for anti-influenza antibody using a commercial NP ELISA (FlockChek™ AI Multi-Screen ELISA, IDEXX Laboratories, Inc.). Receiver operator characteristic analyses were performed on the assay results using MedCalc® 11.3.5 to derive cutoffs and associated sensitivity (Se) and specificity (Sp) estimates. For the analysis, negative status was assigned to unvaccinated, un-inoculated animals and positive status to inoculated ≥ DPI +7 animals. **Results:** A total of 699 known status samples (n=279 negative, n=420 positive) were used to determine the performance of the assay. At an optimized overall cut-off of ≤0.63, the Se and Sp were 95.5% (95% CI: 93.0, 97.3%) and 99.3% (95% CI: 97.4, 99.9%) respectively. By serotype at the overall S/N cut-off of ≤0.63 H1N1 Se was 96.2% (95% CI: 92.6, 98.3%) and

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Sp was 99.3% (95% CI: 97.4, 99.9%); H3N2 Se was 94.8 (95% CI: 90.8, 97.4%) and Sp was 99.3 (95% CI: 97.4, 99.9%). Conclusion: The commercially available nucleoprotein ELISA is an effective assay for influenza antibody detection over time in vaccinated or unvaccinated pigs inoculated with H1N1 and H3N2 influenza virus.

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A predictive model for the detection of influenza antibodies over time using a commercially available nucleoprotein ELISA.

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Purpose: Evaluate the effect of vaccination status, challenge virus, and days post inoculation (DPI) on the detection of serum antibodies in vaccinated or unvaccinated influenza virus-inoculated pigs using a commercial influenza nucleoprotein (NP) ELISA (FlockChek™ AI MultiS-Screen ELISA, IDEXX Laboratories, Inc.). Methods: The study was performed using 82 PRRSV-, SIV- and *Mycoplasma hyopneumoniae*-negative piglets. A subgroup was vaccinated with a multivalent commercial influenza vaccine followed by a booster vaccination 21 days later. All pigs were randomly assigned to one of 3 treatment groups: negative control, Ohio '07 H1N1 inoculated, Illinois '09 H3N2 inoculated. Inoculated pigs received 2ml of virus inoculum ($10^{6.5}$ TCID₅₀/ml) intratracheally. Serum was collected on DPI -42, -21, -7, 0, +7, +14, +21, +28, +35 and +42 and stored at -20°C. At study completion, all serum samples were randomized, and assayed (same day, same technician) for anti-influenza antibody using a commercial NP ELISA (FlockChek™ AI MultiS-Screen ELISA, IDEXX Laboratories, Inc.). Proc GLIMMIX (SAS® 9.2, SAS Institute Inc., Cary NC) was used to analyze a repeated measurement mixed model with PIG nested by VACCINE status as the repeated measure at each bleeding time point. Samples collected DPI ≤0 were categorized by VACCINE (Y/N). Samples from DPI ≥ +7 were classified by TREATMENT (negative control, H1N1, H3N2) and VACCINE status. Results: The Type III test for fixed effects identified DPI, VACCINE, TREATMENT, and the interaction of the three variables as statistically significant to the NP assay S/N results at $p < 0.0001$. Conclusion: Influenza antibody response as detected by the commercial NP ELISA is significantly influenced by vaccination status, inoculum, DPI and their interaction.

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Influenza antibody detection in experimentally inoculated swine over time using two commercially-available indirect ELISAs.

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Purpose: Evaluate two commercial serum ELISAs for influenza antibody detection over time in swine inoculated with a contemporary H1N1 or H3N2 influenza virus. Methods: The study was performed using 82 PRRSV-, SIV- and *Mycoplasma hyopneumoniae*-negative piglets. A subgroup was vaccinated with a multivalent commercial influenza vaccine followed by a booster vaccination 21 days later. All pigs were randomized to one of 3 treatment groups: negative control, Ohio '07 H1N1 inoculated, Illinois '09 H3N2 inoculated. Inoculated pigs received 2ml of virus inoculum ($10^{6.5}$ TCID₅₀/ml) intratracheally. Serum was collected on DPI -42, -21, -7, 0, +7, +14, +21, +28, +35 and +42 and stored at -20°C. At study completion, all serum samples were randomized, and assayed (same day, same technician) for influenza antibody using (a) HerdChek™ H1N1 ELISA (IDEXX Laboratories, Inc), (b) HerdChek™ H3N2 ELISA (IDEXX Laboratories, Inc). Receiver operator characteristic (ROC) analyses (MedCalc® 11.3.5) were performed to derive cutoffs and associated sensitivity (Se) and specificity (Sp) estimates. ROCs were done by serotype, i.e., results from H1N1-challenged animals on the H1 ELISA and H3N2-challenged animals on the H3 ELISA. For the analysis, negative status was assigned to unvaccinated, un-inoculated animals and positive status to inoculated ≥ DPI +7 animals. Results: 489 samples of known status (n=210 positive, n=279 negative) were used to evaluate each assay. At an optimized cut-off of S/P >0.012, the Se and Sp for the H1N1 ELISA were 90.0% (95% CI: 85.1, 93.7%) and 95.3% (95% CI: 92.2, 97.5%), respectively. At an optimized cut-off of S/P >0.005, the Se and Sp for the H3N2 ELISA were 91.9% (95% CI: 87.4, 95.2%) and 95.7% (95% CI: 92.6, 97.8%), respectively. However, at the manufacturer's established cutoff (S/P ≥ 0.4), the H1N1 ELISA Se was 28.6% (95% CI: 22.6, 35.2%) with 100% Sp (95% CI: 98.7, 100%), and the H3N2 ELISA Se was 22.9% (95% CI: 17.4, 29.1%) with 99.6% Sp (95% CI: 98.0, 100.0%). In both assays, over 84% of positives were samples from vaccinates. Conclusion: At the established cut-offs, The H1N1 and H3N2 HerdChek™ ELISAs have limited efficacy in detecting influenza antibody in unvaccinated inoculated swine.

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Novel SYBR real-time PCR assay for detection and differentiation of *Mycoplasma* species in biological samples from various hosts.

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Purpose: Some *Mycoplasma* species are considered significant pathogens in human and animal medicine, while others are considered opportunistic pathogens. *Mycoplasma* sp have fastidious growth requirements and are difficult to detect in clinical samples. We present the development, validation and utilization of a rapid, sensitive, cost effective SYBR real-time PCR (qPCR) detection assay for this genus.

Methods: One set of PCR primers specific for this genus results in PCR amplicons of various lengths dependent on the species present. Sizes and sequence of amplicons determine the melt temperature (TM) and melt profile following High Resolution Melt, thus allowing differentiation of numerous species within this genus occurring in many different hosts or organ systems.

Results: A side by side sensitivity analysis demonstrates equivalent selectivity of the SYBR qPCR assay and conventional nested PCR assay using DNA extracted from bulk tank milk spiked with *M. bovis*. Assay sensitivity levels equivalent to CFU were determined; the qPCR assay was able to detect 1 CFU *M. bovis* in the linear range of sensitivity, has a detection limit of 0.1 CFU and a linear range of detection over 6 logs with PCR efficiency of approximately 90%. Purified stocks of various *Mycoplasma* species were tested in this assay followed by 18s gene sequencing. *Mycoplasma* species included many species found in bovine, caprine, ovine, avian and porcine hosts with greater than 30 species tested to date. 300 plus clinical samples from various hosts and sample types including milk, ocular, ear, nasal and respiratory swabs, joint fluid and reproductive swabs have been tested with this assay commonly yielding equivalent results to other detection methods. Results also include the detection of multiple species from one sample or detection of difficult to culture species as in the case of *M. bovoculi* cattle with pink eye. Conclusion: Here we present a novel real time PCR assay that can detect and characterize all known *Mycoplasma* species tested thus far and provide examples of the utility of this assay in diagnosis, discovery, surveillance, control and eradication of *Mycoplasma* associated disease in multiple host species.

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Genotypic characterization and quantification of *Mycoplasma bovis* in naturally occurring respiratory disease in feedlot cattle.

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Purpose: *Mycoplasma bovis* pneumonia is an important cause of mortality in beef feedlots. The pathogenesis of this condition remains enigmatic because many calves infected with *M. bovis* have no evidence of respiratory disease, suggesting strain-related differences in virulence or the presence of other factors that promote severe disease. This study investigated the relationship of disease status to *M. bovis* concentration and genotypes in naturally occurring cases of respiratory disease in feedlot cattle.

Methods: The study population involved 130 heifers from a single feedlot. Bronchoalveolar lavage fluid (BALF) was collected upon arrival from 60 calves, from all calves with respiratory disease in the first 15 days and a cohort of healthy penmates, and from calves at 60 days post-arrival.

Results: Culture prevalence of *M. bovis* increased from 1.7% on arrival to 74% and 86% at 15 and 60 days post-arrival, respectively; 33, 74 and 100% of calves were seropositive calves at these times. Using real-time PCR, *M. bovis* concentrations in 92 BALF samples obtained at the 3 times ranged from 1.87×10^3 to 1.03×10^8 cfu/ml, but there were no significant differences between calves with and without respiratory disease. Amplified-fragment length polymorphism (AFLP) analysis of *M. bovis* isolates revealed considerable genotypic diversity, with increased diversity at later compared to earlier times. There was no association between AFLP genotype and disease status, in these isolates as well as in a second collection of isolates obtained at necropsy from lungs with caseonecrotic bronchopneumonia and lungs without pneumonia. Different AFLP genotypes were present in some calves at different sampling times, a finding confirmed by analysis of 4 isolates per time point.

Conclusions: AFLP genotypes or *M. bovis* concentration in BALF did not correlate with disease status in this population of feedlot cattle. The AFLP results imply that calves may recover from infection with *M. bovis*, and that re-infection rather than persistent infection may be responsible for the chronicity of this disease.

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Bacterial isolations from lungs of beef calves with bronchopneumonia associated with acute bovine respiratory disease

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Bovine respiratory disease (BRD) continues to be a major problem for the beef cattle industry. Bacterial pathogens such as *Mannheimia haemolytica* and *Pasteurella multocida* are frequently associated with BRD as causal agents of sometimes fatal bronchopneumonia. In this study, 50 lungs, tracheas and tonsils from calves with acute or peracute bronchopneumonia were subjected to extensive diagnostic laboratory investigation to identify and quantitate bacterial pathogens present. The distributions of the bacteria relative to the gross lung pathology were also investigated. Only one bacterial pathogen was isolated from 36% of cases examined and *Mannheimia haemolytica* was the most common pathogen isolated from all tissues. Approximately 50% of the lungs contained $> 10^5$ colony forming units (CFU) per gram of tissue and 12% contained $> 10^8$ cfu. There were significant ($p < .01$) correlations of the species of bacteria recovered between either right or left lungs. Correlations of the isolations between lungs and the tracheal surfaces, major bronchi and tonsillar crypts were significant ($p < .05$) but not as strong as between either right or left lungs. Multi-drug resistant bacteria were isolated from some respiratory tissues but this was not associated with any specific tissue or the density of the bacteria. Viral and mycoplasma pathogens were detected in some tissues but there was no correlation to bacterial species or numbers isolated or to distribution. These results suggest multiple mechanisms of pathogenesis likely contribute to the development of BRD and reinforce the central importance of immunocompetence and stress management of calves.

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Bovine tuberculosis at livestock-human interface in the Pastoralist communities of Southern Ethiopia.

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Globally, tuberculosis (TB) causes 2 millions deaths per year and 8 millions people with disease. The proportion of cases due to *M. bovis* is unknown. Bovine tuberculosis (BTB) is endemic in cross-breed dairy farms and zebu cattle in the central highlands of Ethiopia. The information on human and animal BTB is scarce in pastoral settings. To determine prevalence of BTB in humans and livestock in the pastoralist communities of Oromia and Somali regions in the south Ethiopia, epidemiological study was carried out during 2008-2010. The tuberculin skin testing was conducted on randomly selected 125 herds comprising 59 cattle, 32 camels and 34 goats. A total of 1891 animals with 894 cattle, 479 camels and 518 goats were tested. Abattoir specimens from livestock, sputum samples from humans with pulmonary TB and fine needle aspirates (FNA) from humans with TB lymphadenitis were collected and processed at Armauer Hansen Research Institute. Culture positive isolates were characterized by deletion typing. The prevalence of BTB reactors in tested herds were 4.7% in cattle, 0.4% in camels & 0.2% in goats. The molecular characterization of 29 mycobacterial isolates from livestock revealed that 23 isolates from cattle were all *M. bovis*, of 2 isolates from camels one was *M. tuberculosis* while the other one was non-MTC and all of 4 isolates from goats were non-MTC. Out of 120 isolates from sputum samples 2.5% was *M. bovis* while the remaining was *M. tuberculosis*. This is the most important finding that confirms human-animal transmission of *M. bovis* in this study area. All 6 isolates from FNA samples were *M. tuberculosis*. Results from our study showed that the prevalence of TB due to *M. bovis* was 2.5% in human pulmonary TB and *M. bovis* infection was more prevalent in cattle than in camels and goats. In the future, further detailed study is needed to understand determinants of *M. bovis* transmission between animals and humans.

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Comparative efficacy and immunogenicity of RSV and BRSV vaccines in experimental Bovine Respiratory Syncytial Virus infection.

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Purpose: Bovine respiratory syncytial virus (BRSV) is an important pathogen of young calves, just as human RSV is a cause of severe lower respiratory tract infection in infants. Development of an efficacious and safe vaccine has been problematic for both species. We report the results of a vaccine/challenge study using: placebo vaccine, formalin inactivated RSV vaccine, a modified live commercial BRSV vaccine, and a RSV F protein subunit adjuvanted vaccine.

Methods: 6-8 week old calves were vaccinated and boosted 2 weeks later with either sham vaccine, BRSV MLV commercial vaccine, RSV F

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protein/adjuvant vaccine, or RSV formalin inactivated alum adjuvanted vaccine. One week after the final vaccination calves were infected with virulent BRSV by aerosol or sham infection (placebo vaccine/sham infected negative control group). Clinical signs and virus shedding were evaluated daily. Antibody titers were determined before vaccination, before the booster vaccine, on the day of infection and at necropsy 11 days after infection. Lung pathology and bronchial lymphocyte stimulation responses were evaluated.

Results: Clinical sign scores were significantly different from sham-infected calves, but there was no significant difference between any of the vaccine groups and the placebo vaccine/infected group. The mean final BRSV IFA titer on day 11 post infection was over 2000 for the F antigen/adjuvant vaccine group, and less than 100 for the placebo/infected group. All infected calves shed virus throughout the 10 days of infection. Mean percent lung consolidation at necropsy varied from 0% in the placebo/sham-infected calves to 48% in the group that received commercial BRSV MLV vaccine. Respiratory lymph node lymphocyte proliferation assays to RSV F antigen showed a significant response for all groups except the MLV BRSV vaccinated calves. The response was greatest for the F antigen vaccine group.

Conclusions: This study demonstrates that neither the commercial BRSV vaccine nor two vaccines made with the cross-reactive virus RSV confer protection to experimental BRSV; while all stimulate a strong humoral immune response.

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Impact of route and timing of multivalent respiratory vaccination in the face of maternal antibody (IFOMA) on immune responses to booster vaccination at weaning in beef calves.

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Effective vaccination of calves with circulating maternal antibody to respiratory pathogens is an important goal for cattle producers. Our study evaluated the effect of different routes and timing of priming vaccination IFOMA on immune responses to subsequent booster at weaning in beef calves. One hundred seventy-nine calves were enrolled at 2-4 days of age and randomly divided into five groups (n = 34-37/group). Group 1 (IN Day 2) received modified live virus (MLV) bovine herpesvirus-1 (BHV-1), bovine viral diarrhea virus 1 and 2 (BVDV1/BVDV2), bovine respiratory syncytial virus (BRSV) and parainfluenza type 3 virus (PI3V) vaccine ("MLV 5-way") intranasally (IN) and modified live *Mannheimia haemolytica* (Mh) and *Pasteurella multocida* (Pm) vaccine subcutaneously (SC) at median age day 2. Group 2 (SC Day 2) received MLV- 5-way SC + Mh/Pm SC at d. 2. Group 3 (IN Day 70) received MLV 5-way IN + Mh/Pm SC at d. 70. Group 4 (SC Day 70) received MLV 5-way SC + Mh/Pm SC at d. 70. Group 5 (Control) was not vaccinated until weaning. All calves received MLV 5-way + Mh/Pm SC at weaning (d. 217) and 2 weeks post weaning (d. 231). Serum neutralizing antibody (SN) titers to BHV-1, BRSV, and BVDV1 were measured in all calves; cell mediated immune (CMI) responses to BHV-1, BRSV, BVDV1, and Pm were analyzed in 16 calves per group. Nasal secretions for total IgA and BHV-1 specific IgA were collected from all calves. Samples were collected at median calf age d. 2, 70, 140, 217, and 262. Serum total immunoglobulin concentration (stlg) was measured in all calves at d. 2. For CMI assays, peripheral blood mononuclear cells (PBMC) were isolated and used to assess pathogen-specific interferon gamma (IFN- γ) production, CD25 expression, and cellular proliferation. There was no difference among groups in stlg at d. 2. Vaccinated groups were not higher than controls in nasal total IgA or BHV-specific IgA, in SN titers to BHV-1 or BRSV, or in CMI responses to any agent at the time points assessed. SC Day 70 calves had significantly higher BVDV1 SN titers at d. 217 than all other groups. Priming SC at d. 70 IFOMA led to higher BVDV1 SN titers in calves between d. 70 and weaning, but did not increase titers at weaning or after weaning booster.

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Evaluation of skin samples by RT-PCR following immunization with a modified-live Bovine Viral Diarrhea Virus vaccine.

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Purpose: The objective of this study was to determine if and for how long vaccine virus can be detected using RT-PCR on skin samples following vaccination with a commercially available modified-live BVDV vaccine.

Methods: Two different experiments were conducted in this project. Experiment 1 involved 12 BVDV seropositive steer calves, while experiment 2 involved 7 BVDV seronegative heifers. Skin samples were collected in both experiments at days 0 and 3-18 for virus detection using both individual and pooled RT-PCR. In parallel, blood and nasal swabs were collected for virus isolation.

Results: All skin samples collected from all vaccinated cattle in both experiments were negative for BVDV at all time points using both pooled RT-PCR and individual sample RT-PCR. In experiment 1, BVDV was not isolated from either serum or nasal swabs. In experiment 2, 5 of 7 animals were positive on virus isolation from buffy coats on at least one day and four of seven were positive on virus isolation from serum on at least one day. A representative sample of BVDV isolates from each calf were selected and sequenced. Genetic sequencing showed that the viruses isolated were similar and compatible with the type 1 and type 2 strains contained within the vaccine used in the experiment.

Conclusions: The results of this study provide evidence that it is highly unlikely to detect BVDV in skin samples by either individual or pooled RT-PCR following vaccination with a commercially available modified-live BVDV vaccine. This study supports findings from other studies that have shown similar results using different assay methods. Veterinarians and producers should feel confident that positive test results for BVDV on skin samples is unlikely to be caused by vaccination virus following administration of a modified-live virus vaccine.

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Establishment of a DNA-launched infection cDNA clone for a highly-pathogenic strain of type II PRRSV (strain VR2385): Identification of a naturally-occurring deletion in the nsp2 region that enhances virus replication.

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A highly-pathogenic isolate of PRRSV, ATCC VR2385, was isolated from a pig exhibiting typical PRRS in the early 90's. Pathogenicity studies revealed a highly pneumo-virulent nature of this isolate [Halbur PG, et al. J Vet Diagn Invest. 1996;8:11-20]. During passage of this virus isolate in ATCC CRL11171 and MARC-145 cells, we identified a spontaneous deletion of a 435bp region on the nsp2 gene at passage 8. To evaluate the biological significance of this spontaneous deletion, we first established a DNA-launched full-length genomic cDNA clone of cell culture-adapted VR2385 virus containing the deletion, pIR-VR2385-del. The viral genome engineered with two ribozyme elements at the both ends was placed under the control of the eukaryotic CMV promoter as previously described [Huang YW, et al. Virus Res 2009;145:1-8]. The pIR-VR2385-del was rescued from the transfected BHK-21 cells.

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Inoculation of pigs with the pIR-VR2385-del virus showed that the virus induced characteristic PRRS *in vivo*. Subsequently we constructed another infectious cDNA clone, pIR-VR2385, to restore its parental sequence by introduction of the spontaneously deleted sequence amplified from the passage 4 virus back to the pIR-VR2385-del virus. Comparison of the two viruses (pIR-VR2385-del and pIR-VR2385) by one-step growth curve demonstrated that the pIR-VR2385-del virus with the spontaneous deletion exhibited an enhanced replication (at least 1 log higher) on MARC-145 cells.

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Modulation of host innate immune response by the PRRSV nucleocapsid protein in porcine alveolar macrophages.
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Porcine reproductive and respiratory syndrome virus (PRRSV) infection appears to elicit weak innate immune response suppressing type 1 interferon (IFN) production. Furthermore, several nonstructural proteins including NSP1 α , NSP1 β , NSP2, and NSP11 encoded by the PRRSV genome were demonstrated to independently antagonize the type 1 IFN system. The present study sought to identify the structural proteins that possess the immune evasion properties in porcine alveolar macrophages (PAM). Each structural protein gene was stably expressed in a porcine monocyte-derived macrophage cell line PAM-pCD163 and tested their potentials for mediating the inhibition of IFN production. We then focused on the nucleocapsid (N) protein with a strong inhibitory effect on dsRNA-induced beta IFN production. Upon dsRNA stimulation, the PRRSV N protein was further exhibited to suppress both IFN and IFN regulatory factor 3 (IRF3) promoter activations, indicating the ability of N to modulate the dsRNA-mediated IFN signaling pathways. The N protein significantly interfered with dsRNA-induced phosphorylation and nuclear translocation of IRF3. Our data suggest that the PRRSV N protein is the viral component responsible for evading IFN response by antagonizing IRF3 activation.

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Enhanced activation of swine dendritic cells by NS1-truncated swine influenza viruses.

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Pigs have been hypothesized to act as a 'mixing vessel' which may create novel reassortants of avian, swine, and human influenza viruses capable of causing a human pandemic. The causative agent of current pandemic is triple reassortant H1N1 influenza A virus of swine lineage. The NS1 protein of the influenza virus inhibits type I interferon (IFN) production thus allowing the virus to evade innate host defenses and replicate efficiently. In this study, we conducted in vitro studies to examine the NS1 function of swine influenza viruses using primary swine cells. We used NS1 truncated mutant influenza viruses derived from the swine isolates; Tk/OH/04 (H3N2) and Sw/TX/98 (H3N2) to study the effect of NS1 protein in infected primary cells. The NS1 truncated mutants exhibited reduced viral replication in primary swine respiratory epithelial cells as compared to their wild-type (WT) counterparts. Additionally, swine bone-marrow derived dendritic cells infected with swine NS1 mutant viruses showed higher levels of activation and stimulated naïve T-cells better than WT virus-infected dendritic cells. These results indicate that NS1 truncated mutants are attenuated and induce better activation of dendritic cells.

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Enhanced pneumonia with pandemic 2009 A/H1N1 swine influenza virus in pigs vaccinated with an inactivated δ -cluster H1N2 vaccine.

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Purpose: Inactivated vaccines have demonstrated poor cross-protection against heterologous homosubtypic or heterosubtypic swine influenza A viruses (SIV). The emergence of diverse genetic subtypes has increased the need for effective SIV vaccines with adequate cross-protection. In this report, we describe a vaccine-heterologous virus challenge model demonstrating enhanced lung lesions in pigs given inactivated δ -cluster H1N2 vaccine (human seasonal H1-lineage HA) challenged with heterologous pandemic 2009 A/H1N1 compared to non-vaccinated challenged pigs.

Methods: Twenty-eight vaccinated and challenged (Vx/Ch) pigs were vaccinated at six and three weeks prior to challenge with $10^{5.7}$ 50% tissue culture infectious dose (TCID₅₀) units of UV-inactivated A/SW/MN/02011/08 (MN08) with adjuvant. Vx/Ch and twenty-eight non-vaccinated/challenged (NVx/Ch) pigs were inoculated intratracheally with 2ml of 1×10^5 TCID₅₀/ml A/California/04/2009 H1N1 (pH1N1) at 10 weeks of age. Twenty-eight pigs remained non-vaccinated, non-challenged controls (NVx/NCh).

Results: Pigs in the Vx/Ch group had greater ($P \leq 0.001$) percentages of pneumonia and higher ($P \leq 0.05$) microscopic lung lesion scores compared to the NVx/Ch pigs. Microscopic lesions in each challenge group were similar at 1 dpi but progressively became more severe in the Vx/Ch pigs by 5 dpi. HI and serum neutralizing antibodies to the priming antigen did not cross-react with pH1N1 prior to challenge. Cross-reactive, whole-virus IgG antibodies to pH1N1 were detected in serum and BALF at all time points throughout the study in the Vx/Ch pigs. Virus levels in lung and nasal swabs were significantly higher ($P \leq 0.0001$) in the NVx/Ch pigs at 5 dpi compared to Vx/Ch pigs indicating viral replication alone cannot explain the increase in pneumonia. Conclusions: This study demonstrates an enhancement of pneumonia in vaccinated pigs challenged with a heterologous, homosubtypic virus. This phenomenon has the potential to be realized in the U.S. swine population due to the exposure of genetically diverse H1 SIV currently circulating among swine vaccinated with inactivated heterologous virus vaccines.

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Evaluation of the Newcastle disease virus F and HN proteins in protective immunity using a recombinant avian paramyxovirus type-3 vector in chickens. S. Kumar, B. Nayak, S. Samal, University of Maryland, College Park, MD; P. Collins, National Institute of Health, Bethesda, MD, *Email: sachin22@umd.edu*.

Purpose: Newcastle disease virus (NDV) is an important pathogen of poultry worldwide. Current vaccination strategies are not completely satisfactory. The NDV surface glycoproteins, namely the fusion (F) and the hemagglutinin neuraminidase (HN) proteins, are thought to be the major protective immunogens. However, the relative contributions of F and HN to protection are not well understood. We have developed a recombinant version of avian paramyxovirus serotype-3 (APMV-3) by reverse genetics and have used this recombinant virus as a vector to determine the individual contributions of the NDV F and HN proteins in protection and immunity against NDV in chickens.

Methods: Three recombinant viruses, namely the parental wild-type rAPMV3 vector and rAPMV expressing the NDV F (rAPMV3-F) or HN (rAPMV3-HN) protein were constructed. Protective efficacy was analyzed by vaccinating 2-wk-old SPF chickens by the oculonasal route and challenging on day 21 post-vaccination with virulent NDV via three different routes of inoculation. Serum antibody responses were measured by hemagglutination inhibition and virus neutralization assays.

Results: All three recombinant viruses (rAPMV3, rAPMV3-F, and rAPMV3-HN) protected chickens against NDV challenge via the oculonasal and intramuscular routes, while all unvaccinated birds succumbed to death. This result indicated that rAPMV3 alone can provide substantial cross protection against NDV challenge. However, immunization with rAPMV3 did not protect against intravenous NDV challenge, whereas birds vaccinated with rAPMV3-F alone or in combination with rAPMV3-HN were completely protected, and birds vaccinated with rAPMV3-HN alone were partially protected. NDV neutralizing antibody titers induced by rAPMV3-F were greater than with rAPMV3-HN.

Conclusions: The NDV F and HN proteins are independent protective antigens, but the F protein contributes more to protection than does the HN protein. Wild-type rAPMV3, which is avirulent in chickens, can be used as a vaccine vector against NDV, since APMV3 itself provides protection against NDV due to cross-reactivity and in addition can express NDV protective antigens.

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Cross-protection of pigs vaccinated and challenged with delta-1 and delta-2 sub-cluster H1 Swine Influenza Viruses (SIV).

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Purpose: The most recent endemic swine influenza virus (SIV) H1 cluster to emerge in North America is the δ -cluster of H1N1 and H1N2 viruses that were first reported in 2003-2005. More recently, genetic and antigenic heterogeneity among δ -cluster viruses has been demonstrated. Based on whole genome sequencing and hemagglutination inhibition (HI) cross-reactivity the presence of two sub-clusters, δ -1 and δ -2 has been proposed.

Methods: We evaluated the cross-protection of pigs vaccinated with viruses representing the δ -1 and δ -2 sub-clusters by vaccinating SIV-negative pigs with two doses of killed, adjuvanted experimental SIV vaccines, 2 weeks apart, and challenging 2 weeks later with homologous or heterologous viruses. Vaccine efficacy was evaluated by percent lung lesions at necropsy, virus isolation from nasal swabs and bronchial alveolar lavage fluids (BALF), clinical signs and hemagglutination inhibition (HI) cross-reactivity. The level of significance for all outcomes was $P=0.05$. These studies (Study Report No. 3121W-06-08-682 and No. 3121W-06-09-728, Pfizer Inc) were conducted in accordance with Pfizer Animal Health's IACUC.

Results: Based on HI titers to heterologous viruses, the δ viruses fell into three antigenic groups. In Study 1, vaccination did not reduce lung lesions or virus titers in BALF at necropsy, but did reduce nasal shedding in pigs challenged with a heterologous δ virus. In Study 2 vaccination with the same virus reduced virus titers from nasal swabs and BALF in pigs challenged with a different heterologous virus, although there was no reduction in gross lung lesions. Pigs vaccinated with two other δ viruses were protected from challenge, based on reduction of lung lesions and virus titers in nasal swabs and BALF, but there were differences between the vaccinated groups based on some outcomes.

Conclusions: These challenge data are consistent with previous reports of antigenic differences based on HI cross-reactivity within the δ -cluster of H1 SIV.

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The influence of fetal PCV2 infection on lifetime performance and vaccine efficacy.

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Purpose: With the recent development of porcine circovirus vaccines, porcine circovirus associated disease (PCVAD) has become less of a challenge for swine producers. However, much is unknown about the transmission of the virus from dam to offspring and the effects of viremia at birth on vaccine efficacy. We theorized that viremia at birth would lower vaccine efficacy as measured by growth rate of pigs from birth to 150 days of age.

Methods: In a case control study, serum was collected from 300 sows 21 days prior to and at farrowing. In addition, umbilical cord blood was collected from 10 pigs in each litter and pooled within litter after serum separation. All samples were submitted for qPCR analysis of PCV2 and anti-PCV2 antibodies as measured by IFA to a commercial diagnostic lab. Eight viremic litters were identified and were matched to 8 control litters. Litters were matched based on farrow date, dam parity and antibody titer (at least two-fold higher). Within litter, pigs were blocked to treatment (vaccine, no vaccine) based on birth weight. At weaning pigs were moved to an offsite facility that housed pigs from the same weaning cohort. At weaning and three weeks later pigs were either vaccinated with 2cc of Cirumvent PCV2 (Intervet Schering Plough) or 2 cc of saline. Pigs were weighed at 14, 84 and 154 days of age. Serum samples were collected at 14, 49, 77, 105, 133 and 154 days of age.

Results: Pigs that were viremic at birth were heavier at 14 (4.46 vs. 4.11 kg, SEM 0.08, $p=0.002$), 84 (45.63 vs. 41.89 kg, SEM 0.60, $p=0.001$) and 154 (93.9 vs. 89.9 kg, SEM 1.18, $p=0.020$) days of age compared to non-viremic pigs. Vaccine improved weight gain at 154 days of age by 4.98 kg (94.46 vs 89.4 kg, SEM 1.18 kg, $p=0.003$) but not at other time points. There was no interaction detected between vaccination and viremia at birth. Dams that were viremic at 21 days prior to farrowing were 3.65 (1.27, 10.55, 95%CI) more likely to have a viremic litter. Sow viremia at farrowing was not predictive of piglet infection status.

Conclusions: Based on these data, viremia at birth does not influence vaccine efficacy or lifetime growth under the conditions of this study. Control of sow PCV2 infection is not likely to impact growing pig performance.

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Innate immune responses are enhanced in pigs after sequential infection with influenza virus and *Haemophilus parasuis*.

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Swine influenza virus (SIV) infection alone causes significant disease characterized by respiratory distress and poor growth. However, SIV also plays a significant role in the porcine respiratory disease complex (PRDC), though the mechanism in which this occurs is not clearly defined. *Haemophilus parasuis* (Hps) has been shown to be a complicating factor of influenza in pigs, and both agents continue to be isolated from pigs with pneumonia. After infection, the host immune response is critical for controlling pathogen spread and initiating the adaptive immune response. Tracheal epithelial cells (TEC) and pulmonary macrophages (Mac) are first responders during pulmonary infection with the production of proinflammatory cytokines. Influenza infection is known to predispose to secondary bacterial infection; however, the extent to which antecedent SIV alters TEC and Mac responses to secondary stimulation is poorly understood. To better understand the interaction between SIV and Hps in pigs, groups of pigs were infected with SIV and 5-days later infected with Hps (Flu/Hps). Non-infected, Flu-only and Hps-only groups served as controls. Pigs were necropsied 1 day following Hps inoculation and results show Hps colonization was higher in the nose and lungs of SIV/Hps pigs compared to Hps-only pigs. In SIV/Hps pigs, IL-8, IL-6 and IL-1 β protein levels were increased in the lung, and TEC and Mac cytokine mRNA expression levels were significantly increased over SIV-only and Hps-only pigs. Also, TEC and Mac from 5-day post-SIV pigs exhibited dysregulated innate immune responses to secondary Hps exposure *in vitro*, providing additional evidence that SIV infection alters host responses to secondary stimulation.

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Polymicrobial respiratory disease in pigs.

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Respiratory disease is the most common disease problem in pork production in the U.S. Respiratory disease is most often multifactorial in nature with several combinations of primary and secondary pathogens involved. Pigs are housed in a wide variety of facilities and sizes of populations and they are managed in different ways that also influence the outcome of infection with respiratory disease pathogens. This presentation will review the main viral pathogens commonly associated with the porcine respiratory disease complex (PRDC) including porcine reproductive and respiratory syndrome virus (PRRSV), swine influenza virus (SIV), porcine circovirus type 2 (PCV2), pseudorabies virus (PRV), porcine respiratory coronavirus (PRCV), and porcine cytomegalovirus (PCMV). Viral pulmonary pathogenesis is typically associated with destruction of upper respiratory tract mucociliary apparatus (i.e. SIV, PRV, PRCV, PCMV, PCV2), interference with and decrease of function of pulmonary alveolar macrophages (i.e. PRRSV, PCV2) and pulmonary intravascular macrophages (i.e. PRRSV) and others. Bacterial pathogens such as *Mycoplasma hyopneumoniae*, *Pasteurella multocida*, *Streptococcus suis*, *Actinobacillus suis* and others may also contribute to PRDC mainly by production of inflammation and enhanced cytokine response. In recent years with advancements in pathogen and inflammatory cytokine detection methods, the importance of polymicrobial disease has become more evident and identification of interactions of pathogens and their mechanisms of disease potentiation has become a topic of great interest. For example, the right timing of combined infection with typically low pathogenic agents such as PCV2 and *M. hyopneumoniae* is capable of inducing severe respiratory disease in pigs. Much more needs to be learned about the virulence factors and pathogenesis of common and uncommon swine respiratory disease outbreaks caused by singular and co-infections. In summary, the diagnostic tools are now available to detect and study interactions of various swine respiratory disease pathogens and better understand how they contribute singularly and in combination to clinical disease in pigs.

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A fluorescent bead-based multiplex assay for the simultaneous detection of antibodies to *B. burgdorferi* outer surface protein A (OspA), OspC and OspF in canine serum.

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Purpose: Lyme disease is the most common vector-borne disease in the United States and Europe affecting humans, dogs, and horses and other mammalian species. It is caused by infection with spirochete of the *B. burgdorferi* sensu lato group which are transmitted to the mammalian host by infected ticks (Ixodes). The serological detection of antibodies to *B. burgdorferi* is commonly performed by ELISA which often requires a confirmatory Western blot (WB). Here, we developed a fluorescent bead-based multiplex assay for the detection of antibodies to *B. burgdorferi* in canine serum which combines the benefits of a quantitative ELISA with those of WB in a single test.

Methods: *B. burgdorferi* OspA, OspC and OspF proteins were expressed in *E. coli*. The recombinant proteins were coupled to multiplex beads providing the matrix for the assay. The assay was measured in a Luminex analyzer. The assay was validated against a *B. burgdorferi* WB, the relative gold standard, using two sets of blinded serum samples, 188 sera from patients and 105 sera derived from the first 90 days of controlled infections in 15 dogs using wild caught ticks. The multiplex assay results for each antigen were compared to the corresponding *B. burgdorferi* protein detected by WB using a ROC curve analysis.

Results: A high correlation was observed for detection of antibodies to *B. burgdorferi* in single and multiplex assays resulting in Spearman's rank correlations of 0.93, 0.88 and 0.96 for OspA, OspC and OspF, respectively. The areas under the ROC curves were 0.93 for OspA, 0.89 for OspF, and 0.82 for OspC. Sera from experimentally infected dogs showed that OspC and OspF are useful infection markers and that antibodies were detected as early as 21 days after infection.

Conclusion: The new fluorescent bead-based multiplex assay provides a sensitive and fully quantitative platform for the simultaneous evaluation of antibodies to *B. burgdorferi* OspA, OspC and OspF. The multiplex assay distinguishes between antibodies that originated from vaccination or natural exposure to *B. burgdorferi*.

This work was funded by the Method Development Funds of the Animal Health Diagnostic Center at Cornell University.

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Functional analysis of genes differentially expressed in tick cells in response to *Anaplasma phagocytophilum* infection.

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Pathogens of the genus *Anaplasma* (Rickettsiales: Anaplasmataceae) have evolved molecular mechanisms which facilitate their development in tick vectors. We recently demonstrated that *A. marginale* infection in tick cells affects the expression of tick genes which are essential for pathogen infection and multiplication and tick survival. The biological significance of many genes, as well as their role in pathogen-tick host cell interactions, has not been defined. In this study we used proteomics and real time RT-PCR to evaluate the role of genes differentially expressed in cultured ISE6 tick cells in response to *Anaplasma phagocytophilum* infection. Monolayers of cultured *Ixodes scapularis* cells (ISE6) were infected with *A. phagocytophilum* and then collected during early and late infection. Differentially-expressed proteins in the infected vs. uninfected cells were then identified. Genes encoding for the differentially-expressed proteins involved in cell growth, differentiation and transport metabolic pathways were then characterized during early and late infection by real time RT-PCR. Genes that exhibited significant differences in the mRNA levels were subsequently selected for evaluation by RNA interference (RNAi) in order to test the effect of gene silencing on pathogen infection levels as determined by *msp4* PCR. During infection with *A. phagocytophilum* 15 metabolically important proteins were found to be differentially-expressed in the cultured tick cells. Some genes were downregulated while others were upregulated. Of these, 6 genes were found to be significantly different at the mRNA level. When subjected to gene knockdown by RNAi, two genes encoding for a spectrin alpha chain cytoskeletal protein and a voltage-dependent anion-selective channel mitochondrial protein resulted in significantly lower or higher *A. phagocytophilum* infection levels, respectively, when compared with controls. This functional proteomic and genomic approach allowed for discovery of genes that play a role in pathogen/tick host cell interactions.

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Survey of *Ehrlichia* and *Anaplasma* species in white tailed deer by real-time RT-PCR or PCR and DNA sequence analysis.

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Purpose: *Ehrlichia* and *Anaplasma* species are rickettsial organisms which infect a variety of mammalian species. The organisms are transmitted from ticks and are maintained in reservoir hosts. Several pathogens have been identified in recent years as the causative agents for emerging infections in people. One of the primary reservoir hosts for the pathogens is the white tailed deer. This molecular evaluation study was conducted to assess the rate of prevalence of *Ehrlichia*/*Anaplasma* agents in white tail deer.

Methods: In this study, 147 deer blood samples and 37 ticks were evaluated for the prevalence of *Ehrlichia*/*Anaplasma* species by TaqMan-based real time amplification assay and DNA sequence analysis.

Results: Among 147 blood samples, 114 (74%) samples tested positive with the *Ehrlichia*/*Anaplasma* genera-specific probe. They included 75 positives for the DNA and RNA targets, 22 DNA test positives and 16 RNA test positives. Further analysis of the samples with the probes specific for human ehrlichiosis agents, *E. chaffeensis* and *E. ewingii* identified 4 (2.7%) and 7 (4.7%) positives, respectively. Test positives from 24 randomly selected samples were further evaluated by sequence analysis targeting to a 426 bp segment of 16S rRNA gene. All 24 samples were confirmed as positive for the *Anaplasma* and *Ehrlichia* white-tailed deer isolates, WTD81 (GenBank #DQ007352.1) and GA isolate # 4 (GenBank #U27104.1) (both of which are nearly identical for the sequences analyzed with one base pair difference). DNAs from 37 pools of ticks (1 to 2 ticks) collected from the white tailed deer were also evaluated. The TaqMan-based real time PCR assay with *Anaplasma*/*Ehrlichia* common probe identified 29 (78%) tick pools as positives whereas *E. chaffeensis*- and *E. ewingii*-specific probes identified three (8%) and one (3%) positives, respectively.

Conclusions: The high prevalence of deer isolates and the presence of human ehrlichiosis pathogens in deer are similar to earlier findings. These data demonstrate the need for further assessment of the pathogenic potential of the deer isolates to people and domestic animals.

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Preliminary development of an approach to rapidly identify and isolate *Ehrlichia chaffeensis* from naturally infected domestic hosts.

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Preliminary development of an approach to rapidly identify and isolate *Ehrlichia chaffeensis* from naturally infected domestic hosts.

Human monocytic ehrlichiosis (HME) is a tick-borne zoonosis that significantly affects human health. White-tailed deer and *Amblyomma americanum* ticks, both of which are indigenous throughout Missouri, are considered the primary reservoir and vector of *Ehrlichia chaffeensis*, the etiologic agent of HME. The consequence of natural transmission of *E. chaffeensis* among domestic hosts is poorly understood. Previous studies indicated that *E. canis* can be isolated from carrier blood within 24 hours of collection, but that longer periods of refrigeration reduced the infectivity of these organisms for cell culture. The purpose of this project was to adapt a PCR assay for rapid detection of *E. chaffeensis* to facilitate isolation of *E. chaffeensis* from naturally infected blood samples. A universal 16S rDNA-based real-time PCR assay for tick-borne members of the rickettsial family Anaplasmataceae was compared to outer membrane protein gene based real-time PCR assays that are specific for *E. chaffeensis* and *E. canis*. These assays were also compared for screening canine and feline blood samples provided by the Clinical Pathology Section of the University of Missouri Veterinary Medical Diagnostic Laboratory. Preliminary results will be reported, and the strategy for identification and isolation of *E. chaffeensis* from naturally infected domestic hosts will be discussed.

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Utilization of a peptide based enzyme linked immunosorbant assay for the detection of bovine anti-*E. chaffeensis* antibodies.

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Ehrlichia chaffeensis is an obligate intracellular prokaryotic parasite of monocytes and macrophages, and the causative agent of human monocytic ehrlichiosis (HME). *E. chaffeensis* is thought to be maintained in a complex life cycle involving the white-tailed deer (WTD) as a reservoir, and *Amblyomma americanum* as a vector. The large distribution of WTD, and the aggressive feeding habits of all stages of *A. americanum* on medium and large sized mammals makes it probable that additional species are exposed. PCR has been used to demonstrate natural infection of dogs, coyotes, goats, and lemurs. Serologic methods have also detected antibody responses to *E. chaffeensis* in raccoons, opossums, foxes, and rabbits. Dairy cattle have been shown to be experimentally susceptible to *E. chaffeensis* infection that resulted in mild to severe clinical signs and pathologic lesions similar to those seen in HME. Cattle ranges overlap with WTD and *A. americanum* making it likely that they are naturally exposed. However, information is lacking on what role these ruminants may play in the ecology of *E. chaffeensis*, or the impact this pathogen may have on this population.

Purpose: We hypothesize that cattle are susceptible to natural infection with *E. chaffeensis*. The objective of this study was to develop a peptide based enzyme linked immunosorbant assay (ELISA) for detection of anti-*E. chaffeensis* antibodies in cattle.

Methods: Cattle were intravenously inoculated with *E. chaffeensis* infected DH82 cells. Plasma samples from the experimentally infected cattle were used for the optimization of ELISAs utilizing various peptides of outer membrane proteins of *E. chaffeensis*. The ELISA was optimized for plasma, secondary antibody, and peptide concentrations through evaluation of optical density.

Results: The bovine ELISA was able to detect anti-*E. chaffeensis* antibodies in cattle, and optimization of assay parameters increased overall sensitivity.

Conclusions: The use of this bovine *E. chaffeensis* ELISA may be useful in characterizing immune responses of experimentally infected cattle or as a screening tool to identify natural infection.

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The *Index Catalogue of Medical and Veterinary Zoology*, the quintessential parasitology literature research tool, now digitized, preserved, and freely available worldwide.

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The *Index Catalogue of Medical and Veterinary Zoology*, beginning in 1892, was created by Charles Wardell Stiles and Albert Hassall, parasitologists working for the Bureau of Animal Industry, United States Department of Agriculture. This series, a key resource, is a historical compendium of the parasitological literature and of importance to researchers in re-emerging diseases and global animal health. By the time publication was suspended in 1982, the compilation resulted in more than 100 separate issues comprising more than 20,000 pages. Originally published as the *U. S. Bureau of Animal Industry Bulletin Number 39* in 1902-12, the author portion of the catalogue was subsequently issued as a series of government documents available from the Superintendent of Documents, beginning in 1932. From 1932 to 1952, the process moved systematically through the author alphabet, until number 18 was issued (authors' names "X" - "Z"). Publication was expanded to include subject portions of the catalogue and supplements were issued periodically. Like the author catalogue, all portions were available from government sources for a nominal fee until the early 1980s. When government publication ceased, Oryx Press undertook publishing material already assembled by government employees. That effort resulted in Supplement #24, produced in several parts; when that effort ended in 1982, publication was suspended. In 2008, librarians at the Texas A&M University and Oklahoma State University submitted a grant application to the South Central Region of the National Network of Libraries of Medicine, which was funded for \$20,000, enabling digitization and preservation of the catalogue. Scanning of the documents to tiff and conversion to pdf/a was completed in Summer 2010. The entire catalogue issued as U. S. Government Documents should soon be available electronically, and free of charge from both university library websites. Efforts are underway to obtain copyright permission for the portion issued by Oryx Press so that it can also be released. The digitized catalogue is full-text searchable and has standard metadata assigned.

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Bovine Anaplasmosis: An Overview of current challenges.

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Bovine anaplasmosis, caused by the obligate intracellular rickettsia *Anaplasma marginale*, is endemic in several areas of the United States and has continued to impact cattle production. The dynamics of the epidemiology of *A. marginale* are more complex than other tick transmitted diseases of cattle because transmission occurs both biologically by ticks and mechanical transmission occurs iatrogenically by blood contaminated needles and veterinary instruments or by blood contaminated mouthparts of biting flies. While much research has been done on *A. marginale* over the past several decades, control strategies for anaplasmosis have advanced minimally since the first vaccine was marketed in the US in the 1960s. However, these research findings have continued to contribute to our overall understanding of the complexity of bovine anaplasmosis and have advanced our understanding of the biology of the pathogen and its relationship with cattle and tick hosts. The classification of *Anaplasma* was reorganized to include several organisms in addition to those that are host-specific for ruminants, including the emerging tick-borne pathogen of humans, *Anaplasma*

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phagocytophilum. Even though bovine anaplasmosis is a recognized disease in the United States, the epidemiology of anaplasmosis is complex and not well understood. The diversity of *A. marginale* strains is far greater than previously recognized, especially in areas of the U.S. with extensive cattle movement. Advances have been made in understanding the developmental cycle of *A. marginale* in cattle and ticks, defining the role of male ticks in transmission of anaplasmosis and defining the molecular interactions of the tick-pathogen interface. New information based on molecular studies of wildlife reservoirs of *A. marginale* has recently been reported and may warrant reinvestigation of wildlife hosts of *A. marginale*. The potential influence of climate change on the epidemiology of anaplasmosis will be discussed and the current status of anaplasmosis vaccines will be presented. Dual target vaccine strategies that target control of tick infestations and reduce the capacity of ticks to serve as a vector are currently being developed.

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Detection of *E. chaffeensis* in post-mortem blood and tissue samples from an elk using real-time PCR.

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Ehrlichia chaffeensis is a vector-borne bacterium with a tropism for monocytes and macrophages, and is the causative agent of human monocytic ehrlichiosis (HME). The primary mammalian reservoir of *E. chaffeensis* is considered to be the white-tailed deer, and the primary vector *Amblyomma americanum*. Antibodies reactive with *E. chaffeensis* have been reported from mule deer in Arizona; however, no molecular evidence of *E. chaffeensis* was detected. PCR has been used to detect *E. chaffeensis* in marsh deer in Brazil, sika deer in Japan, and a spotted deer in Korea. In California, other cervids including mule deer, black-tailed deer, and elk have been assayed with PCR and IFA for the detection of *E. chaffeensis*, but were found to be negative.

Purpose: The objective of this study was to detect *E. chaffeensis* in blood, tissue, and tick samples collected from an elk.

Methods: Blood, tissue, and tick samples were collected postmortem from an elk submitted to the University of Missouri Veterinary Medicine Diagnostic Laboratory. The buffy coat (BC) was manually removed from the blood sample, and DNA extracted from it and the other sample types for PCR analysis. A real-time PCR assay based on an outer membrane protein of *E. chaffeensis* was used to assay the various specimens. BC DNA was also assayed with a conventional PCR assay based on the *p28* gene of *E. chaffeensis*. BC samples amplified with PCR were sequenced at the University of Missouri DNA core. Results: Ticks removed from the elk were visually identified as *Dermacentor* spp. and were found to be PCR negative. BC and spleen DNA samples were PCR positive for *E. chaffeensis*. Sequence results from the *p28* PCR amplicons of BC samples were most similar to the *E. chaffeensis* V2 isolate with which it shared an 85% identity.

Conclusions: To our knowledge, this is the first description of natural *E. chaffeensis* infection in an elk. This finding highlights the need for continued surveillance of cervid populations for the presence of *E. chaffeensis* and examination of their role in the complex ecology of this pathogen.

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The effect of anthelmintics on proinflammatory cytokine responses in treated horses.

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Larval cyathostomiasis is a condition in horses that can often lead to death. Caused by a sudden emergence of small strongyle larvae from encysted stages in the large intestine, it can be prevented through use of anthelmintics. Somewhat paradoxically, administration of anthelmintics can also be associated with ulcers and acute inflammation in the intestinal tissues. This inflammatory response was most noted after treatment with larvicidal drugs and likely represents the host responses to degenerating larvae in the tissues. To test this hypothesis, we treated three groups of horses treated with pyrantel tartrate, fenbendazole or moxidectin and monitored their inflammatory cytokine expression post-treatment. Twenty horses, between one and three years of age and naturally infected with cyathostomins and other parasites after pasture grazing, were used in the study. The horses were divided into four equal treatment groups such that group 1 received no de-worming treatment, group 2 received 5 daily doses of fenbendazole (10 mg/kg), group 3 received daily treatment of pyrantel tartrate 2X (2.65 mg/kg), and group 4 received moxidectin (400 µg/kg). Peripheral blood sample were collected into a PAXgene™ blood RNA tube daily for two weeks. Fecal samples were collected for parasitological examination. The objective of this study was to determine the effect of de-worming on pro-inflammatory cytokine gene expression. While fenbendazole is known to induce an inflammatory response and moxidectin is known to exhibit anti-inflammatory activity, we expect to see reduced evidence of inflammatory responses to treatment with pyrantel tartrate, given the chemical's ability to prevent larval encystation.

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Purification of recombinant anaplasma appendage associated protein for studies that involve understanding its biological activity.

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Purification of recombinant anaplasma appendage associated protein for studies that involve understanding its biological activity. Bakul Dhagat-Mehta¹, Roger W. Stich¹. Dept of Veterinary Pathobiology, College of Veterinary Medicine, University of Missouri, Columbia, MO
Bovine anaplasmosis is considered the most widespread vector-borne disease of livestock worldwide. *Anaplasma marginale*, the primary agent of bovine anaplasmosis and a model organism for the rickettsial family Anaplasmataceae produces anaplasma appendage associated protein (AAP). Anaplasma appendage associated protein is associated with F-actin within macromolecular structures known as inclusion appendages. These structures are also found in the tick blood meal suggesting that actin-based motility may facilitate *Anaplasma marginale* contact with tick midgut epithelium. Despite the potential importance of AAP in manipulation of host actin, the role of this protein in *A. marginale* transmission and infection remains unclear.

Towards this end we intend to understand how AAP interacts with the erythrocyte skeleton. Open reading frames encoding the polymorphic AAP sequences were optimized for Escherichia coli codon usage and cloned into pGS-21a vector. The pGS-21a vector is designed for cloning, high-level expression and convenient purification of proteins fused with both 6xHis and GST. These tags can be cleaved using enterokinase. The recombinant AAP has been overexpressed using IPTG and found to be in inclusion bodies. The inclusion bodies were then denatured and recombinant AAP was refolded using 6M Urea. The refolded recombinant AAP was then affinity purified and the tags were successfully cleaved off using recombinant porcine enterokinase. Future work involves using this purified fusion protein to evaluate the eukaryotic binding partners of AAP. Once the binding partners are known their presence can be confirmed within the appendages. Once we understand the AAP-cytoskeleton interaction it will become possible to develop an *in vitro* model to investigate parameters of the biologic activity of AAP and its potential role(s) in *A. marginale*

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Replacement of the replication factors of Porcine Circovirus (PCV) Type 2 with those of PCV Type 1 greatly enhances viral replication *in vitro*.
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Purpose: Porcine circovirus type 1 (PCV1), originally isolated as a contaminant of PK-15 cells, is nonpathogenic whereas porcine circovirus type 2 (PCV2) causes an economically-important disease in pigs. To determine the factors affecting virus replication, we constructed four novel chimeric viruses by swapping ORF1 (*rep*) or the origin of replication (*Ori*) between PCV1 and PCV2, and compared the replication efficiencies of the chimeric viruses in PK-15 cells.

Methods: A one-step growth curve was performed at 0.5 multiplicity of infection for each virus, and infectious titers were measured in triplicate every 12 h through 96 h post-infection (hpi) using an immunofluorescence assay (IFA).

Results: PCV1 replication was significantly higher than PCV2 from 36 hpi through the end of the study ($p \leq 0.005$), with the replication of the chimeric viruses intermediate to the two parental viruses.

Conclusions: The results demonstrate that the replication factors of PCV1 and PCV2 are fully exchangeable, and most importantly, that the *Ori* and *rep* of PCV1 each enhance virus replication efficiency of the chimeric viruses with PCV2 backbone.

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A chimeric Porcine circovirus (PCV) with the capsid gene of PCV2b cloned in the genomic backbone of PCV1 is attenuated *in vivo* and protects pigs against PCV2a and PCV2b challenge.

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Purpose: Porcine circovirus type-2 (PCV2) is the primary causative agent of porcine circovirus-associated disease (PCVAD). Recently, a new PCV2b subtype associated with more severe clinical PCVAD has become the dominant strain in swine herds globally. The current commercial vaccines are all based on the PCV2a subtype, and thus it is important to develop a novel vaccine against the emerging PCV2b subtype.

Methods: In this study, we first constructed a chimeric virus (designated PCV1-2b) with the PCV2b capsid gene cloned into the backbone of non-pathogenic PCV1. After demonstrating that the PCV1-2b is viable *in vitro*, a pathogenicity study was conducted with 30 caesarean-derived colostrum-deprived pigs. To evaluate the chimeric PCV1-2b as a vaccine, we subsequently conducted a challenge study: 40 conventional pigs were vaccinated with chimeric PCV1-2b (n=20) or left unvaccinated (n=20), and subsequently 10 pigs in each group were challenged with PCV2a and PCV2b, respectively, to determine protective and cross-protective immunity.

Results: The results of the pathogenicity study showed that pigs inoculated with chimeric PCV1-2b (n=10) had decreased lymphoid lesions and significantly lower viral load at 21 dpi, and significantly lower viremia starting at 14 dpi compared to pigs inoculated with PCV2b (n=10), which developed characteristic PCVAD. The results of the challenge study showed that vaccination with the chimeric PCV1-2b protected pigs against PCV2b challenge and PCV2a cross-challenge. Vaccinated pigs had no detectable viremia and significantly decreased overall lymphoid lesion scores and lower viral loads compared to unvaccinated controls.

Conclusions: The chimeric PCV1-2b virus is fully attenuated, and is a good candidate for a live vaccine against both PCV2b and PCV2a subtypes.

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Transmission of PCV2 from sow to piglet in the farrowing room.

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Porcine circovirus 2 (PCV2) infection, which is necessary for PCV-associated disease (PCVAD), is widespread in swine farms throughout the United States. Vaccination of pigs, frequently around the time of weaning, has been effective in preventing PCVAD and reducing the level of PCV2 in serum, but it does not eliminate infection. Since nearly all of the finishing herds in the USA are vaccinated, we are inadvertently providing a large-scale selective pressure on PCV2 for new strains that grow better in the presence of an anti-PCV2 vaccine response. Improving vaccine efficacy against infection requires a better understanding of when and how pigs are exposed to and become productively infected with PCV2. Infection is thought to occur in finishers at 10-15 weeks of age, when they become viremic. However, infection of sows may be prevalent, and PCV2 is known to be shed in colostrum, milk, and feces, and the virus is stable in the environment. Therefore, we hypothesized that piglets are exposed to PCV2 at or before birth and throughout the suckling period, and that anti-PCV2 antibodies in colostrum might suppress viremia. We examined sows, pre-suckling piglets, and the farrowing environment of sow farms for PCV2 virus and PCV2-specific antibodies. PCV2 DNA was observed in serum, oral fluids, and colostrum of sows, even though antibody levels were high in serum and colostrum. PCV2 DNA was detected in tissues from stillborn pigs and mummified fetuses, and in serum of pre-suckling liveborn piglets, indicating that piglets are readily infected with PCV2 in utero. PCV2 also was detected on axillary skin of presuckling pigs and sows and on farrowing crate surfaces. Farrowing crate disinfection procedures reduced, but did not eliminate detection of viral DNA. Overall, PCV2 is widely distributed in sow farms and is transmitted to piglets in utero and after birth. The presence of high levels of antibodies does not resolve infection in sows, but may suppress infection in piglets since absence of viremia is commonly observed in nursery-age pigs.

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Diversity of porcine torque teno viruses.

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Purpose: Our objective is to establish diagnostic tools, generate infectious clones, and to study diversity of porcine torque teno viruses (PTTV) which become increasingly important in swine production.

Methods: Seven type I and six type II PTTV strains were cloned and sequenced to study their diversity and phylogenetic relationships.

Results: Porcine torque teno viruses (PTTV) are about 2.8-2.9kb, and has been considered to enhance disease severities in pigs infected with PCV2 and/or PRRS. A duplex real-time PCR test is generated to monitor PTTV in PCV2 and PRRS positive diagnostic samples. From a PTTV positive diagnostic sample, two full genomes of type I PTTV, PTTV139-2 and PTTV139-8, were cloned and sequenced. They are 2.9kb, sharing 69% DNA identity, however, sharing higher identity in the first 705bp (86%), and a 590bp GC-rich region (93%) in the end of the genomes. Interestingly they carry a ~1.6kb region in the middle of the genomes that is not related to each other. From a separate diagnostic sample, a few dozens of 2.4kb fragments without the GC-rich region were cloned to further study the diversity of type I PTTV by restriction mapping. Digestion with *EcoRI*, *PstI*, and *KpnI/SacI* (double digestion) identified five different type I strains in this diagnostic sample. They are 96-99% identical, but 59% to PTTV139-8, and 56% to PTTV139-2. Six type II PTTV (PTTVII)

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strains, two each from three swine diagnostic samples, were also cloned and sequenced. Two strains are identical, and are 91-92% identical to other three strains, and 76% identical to the sixth strain which appear to be more different among the six cloned PTTVII strains. The six PTTVII strains are 37-39% identical to PTTV139-2 and PTTV139-8, and are 33-37% identical to the 2.4 kb partial genomes. Phylogenetic analysis shows that the type II strains formed a distinct branch. PTTV139-8 is in the cluster closer to the 2.4 kb type I strains than the cluster of published type I strains. PTTV139-2 is close but different from the published type I strains.

Conclusions: PTTV is very diverged. Multiple strains were isolated from the same animal. Their role in PRRS and/or PCV2 positive animals will be further studied once infectious clones are generated.

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Development of SYBR green-based real-time PCR assays for quantification and differential detection of species-specific porcine Torque teno viruses (TTV)
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Porcine Torque teno virus (TTV) is a single-stranded circular DNA virus that has recently been incriminated in swine diseases. Multiple infections with porcine TTV species 1 (PTTV1) and species 2 (PTTV2), each consisting of two types (PTTV1a and 1b) or subtypes (PTTV2b and 2c), in a single pig had been recently discovered by our group (Huang YW et al., *Virology*, 2010, 396:289-297). Here we describe two novel assays for quantification and differential detection of porcine TTV. Firstly, we developed two SYBR green-based real-time PCR assays to specifically quantify viral loads of the two porcine TTV species, respectively. The PTTV1- and PTTV2-specific real-time PCR primer sequences were selected to target conserved genomic regions identified by multiple sequence alignments of 10 available porcine TTV full-length genomes. Secondly, by coupling the two singleplex PCR assays, we determined that a duplex real-time PCR assay followed by melting curve analysis was also established allowing for simultaneous detection and differentiation of PTTV1 and PTTV2. These assays provide rapid and practical tools for molecular diagnostics of species-specific porcine TTV infection in pigs.

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Serodiagnosis of porcine Torque teno virus (TTV) infection in pigs by Western blot and indirect ELISAs

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Porcine Torque teno virus (TTV) with a circular DNA genome of ~2.8 kb resembles the genomic organization of human TTV-related viruses that are currently classified into a newly-established family Anelloviridae. Porcine TTV has a high prevalence in both healthy and diseased pigs worldwide, as determined by conventional PCR, and has been shown to partially contribute to porcine diseases when pigs were co-infected with other porcine viral pathogens such as PRRSV and PCV2 in the United States. We had recently identified four full-length genomic sequences of porcine TTV representing two different species (PTTV1 and PTTV2), each consisting two genotypes (PTTV1a and 1b) or subtypes (PTTV2b and 2c), from a single serum sample, implying that, for the first time, multiple porcine TTV infections with distinct genotypes or subtypes exist and probably are common in pigs (Huang YW et al., *Virology*, 2010, 396:289-297). However, the prevalence of specific porcine TTV antibody levels in pigs remains unknown. In this study, the putative capsid (ORF1) proteins from PTTV1a, 1b and 2c strains fused with histidine tags were expressed in *E. coli*, respectively. The purified recombinant proteins were used as the antigens for the detection of type-specific PTTV IgG antibodies in pig serum samples by Western blot. The positive and negative pig sera were subsequently used as the standards for the development of three type-specific indirect ELISAs for serological diagnosis of porcine TTV. The results revealed a high rate of seropositivity to PTTV in most pigs from different sources including CD/CD pigs. However, gnotobiotic pigs tested from Virginia and Iowa were seronegative. A retrospective analysis of 10 conventional pigs during a two-month period showed that decreased viral loads or virus clearance were associated with elevated anti-ORF1 IgG antibody levels, suggesting that the TTV ORF1 indeed encodes a viral structural protein and may contain neutralizing epitopes. Our study established essential serodiagnostic tools for the investigation of porcine TTV, which will help elucidate the potential pathogenicity of porcine TTV infection in pigs.

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Interaction between structural Core protein of Classical Swine Fever Virus with IQGAP protein appears as essential for virus virulence in swine

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Structural components of the Classical swine fever virus (CSFV) virion include the Core protein and glycoproteins Erns, E1, and E2. CSFV nucleocapsid (Core) protein has been shown to act as a regulator of transcription in addition to its protective function for the viral RNA. However, studies involving the CSFV Core protein have been limited. To gain insight into other functions of the Core protein, particularly into its possible role in virus virulence, we utilized the yeast two-hybrid system to screen a custom swine primary macrophage cDNA library to identify host proteins that physically interact with the viral Core protein. Our studies revealed specific binding of several host proteins to the viral Core protein. One of the identified proteins is a important regulator of the cytoskeleton: IQGAP1. IQGAP1 has been implicated in many aspects of cell physiology as intracellular transport and cell adhesion. It has been already described that Moloney murine leukemia virus (MMLV) matrix also binds IQGAP1 protein (EMBO J, 2006 25:2155). Based in the sites of MMLV matrix protein that recognize IQGAP four (I-IV) areas in CSFV Core protein were identified. Substitution of Core native amino acid residues to alanine in areas I and III abolish CSFV Core IQGAP interaction. CSFV mutants harboring substitutions in these four areas were developed to assess the importance of Core-IQGAP interaction for viral virulence in swine. Remarkably, substitutions in areas I and III in the Core protein affecting IQGAP recognition correlate with complete absence of virulence in swine. Therefore, the ability of CSFV Core to bind host IQGAP protein during the infection may play a critical role in virus virulence.

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The effect of glycosylation on antigenicity and immunogenicity of Classical Swine Fever Virus envelope proteins.

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Glycosylation of viral envelope proteins is a common form of post-translational modification which could be essential for correct folding, transport, secretion, as well as other functions including virus assembly, virus binding, and virus entry into host cells. Classical Swine Fever Virus (CSFV) encodes

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three envelope glycoproteins, namely, E^{ms}, E1, and E2. E2 glycoprotein is immunogenic and has been used for development of candidate vaccines against the virus. While CSFV E2 protein has been extensively studied, little is known about the immunogenicity of E^{ms} and E1, and the potential role of N- and O-glycosylation in influencing the immunogenicity of these three viral envelope proteins. Previously, we have shown that viruses carrying mutations of specific N-glycosylation sites on E^{ms}, E1 and E2 are attenuated in swine and could protect animals from challenge with virulent CSFV. To further analyze these effects we investigated first whether the complete lack of glycosylation of baculovirus-expressed E^{ms}, E1, and E2 proteins affects the induction of humoral immune response in pigs. Nonglycosylated E^{ms} and E2 failed to induce a long-lasting antibody response and did not protect pigs against challenge with virulent CSFV. Only pigs vaccinated with baculovirus-expressed glycosylated E^{ms} and E2 proteins, but not with glycosylated or nonglycosylated forms of E1 protein, were protected against challenge infection. To assess the effect of individual glycosylation sites on E^{ms} and E2 antigenicity and immunogenicity, we expressed mutant E^{ms} and E2 proteins using the baculovirus/insect cells expression system. Swine were then inoculated with each mutant protein and antibody response and survival to challenge were analyzed.

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Bovine viral diarrhea virus fetal infection following immunization with a contaminated modified-live virus vaccine.

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The aim of this study was to determine whether a multivalent modified-live virus (MLV) vaccine containing noncytopathic (ncp) bovine viral diarrhea virus (BVDV) administered off-label to pregnant cattle can result in persistently infected (PI) fetuses and assess whether vaccinal strains can be shed to unvaccinated pregnant cattle. Nineteen BVDV-naïve pregnant heifers were randomly assigned to two groups: cattle vaccinated at day 75 of gestation with a MLV vaccine containing BVDV-1a (WRL strain), BoHV1, PI₃, and BRSV (Vx; n=10) or control unvaccinated cattle (n=9). During the course of the study a voluntary stop sale/recall was conducted by the manufacturer due to the presence of a BVDV-2 contaminant in the vaccine. At day 170 of gestation, fetuses were removed by caesarean section and fetal tissues were submitted for virus isolation, and qRT-PCR using BVDV-1- and BVDV-2-specific probes. Sequencing of viral RNA was performed for qRT-PCR positive samples. Two vaccinated and two control heifers aborted, but their fetuses were unavailable for BVDV testing. BVDV was isolated from the all 8 fetuses in Vx and from 2 of 7 fetuses in the control group. Only BVDV-2 was detected in fetuses from Vx, whereas only BVDV-1 was detected in the 2 fetuses from the control group. Both BVDV-1 and BVDV-2 were detected in the vaccine. In conclusion, vaccination of pregnant heifers with contaminated modified-live BVDV vaccine resulted in the development of BVDV-2 PI fetuses in all the tested vaccinated animals. BVDV was shed to unvaccinated heifers causing fetal infections from which only BVDV-1 was detected.

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A Bovine Viral Diarrhea Virus type 1b subunit vaccine induces neutralizing antibodies in calves.

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Purpose: Bovine viral diarrhea virus (BVDV) type 1b is wide spread and the predominate type circulating in the US cattle population. Recent studies demonstrated that limited or no cross neutralizing activity occurred when using antisera generated against types 1a, 1b and 2 BVDV. At the present time, most of the commercial vaccines do not appear to induce protection against type1b strains of BVDV. In this study, we evaluated a type 1b BVDV subunit vaccine.

Methods: The envelope protein E2 of BVDV is a major antigen involved in inducing neutralizing antibodies. The E2 gene of BVDV was cloned into a transfer vector and recombinant baculovirus expressing E2 protein of BVDV1b was generated. To evaluate its protective role, 20 eight week-old calves were divided into groups of five each and two of the groups were vaccinated twice with the extracts of inactivated recombinant baculovirus expressing BVDV1b E2 protein. Blood serum was collected from the enrolled calves on Day 0, 21 and 35 post first vaccination and was evaluated for the presence of virus neutralizing antibody using a SN assay.

Results: The sera from vaccinated calves had SN titers of 16 to 512 (GMT 181) and control group stayed negative with titers <1: 8 for BVDV1b. The sera showed some cross reactivity with type1a and type2 strains of BVDV. Further studies are underway to determine the duration of response and protective immunity in calves.

Conclusions: In conclusion, we demonstrated that a subunit vaccine using an inactivated recombinant baculovirus expressing BVDV1b E2 protein elicits neutralizing antibody in calves.

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Bovine viral diarrhea virus N^{pro} antagonism of IFN-I mediates enhancement of bovine respiratory syncytial virus replication during co-infections.

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Concurrent infections with bovine viral diarrhea virus (BVDV) and bovine respiratory syncytial virus (BRSV) cause more severe respiratory and enteric disease than infection with either virus alone. The BVDV genome encodes an autoprotease, N^{pro}, that degrades interferon regulatory factor-3 (IRF-3) reducing type I interferon (IFN-I) production by host cells. Purpose: We hypothesized that N^{pro} modulates the innate immune responses to BVDV infection and enhances BRSV replication during co-infection. The objectives of this study were: (1) to characterize the replication kinetics and IFN-I induction in cells following viral infection, and (2) to characterize the influence of BVDV-mediated IFN-I antagonism on enhancement of BRSV replication. Methods: Noncytopathic BVDV type 2 wild-type (wt) NY93, infectious clone NY93/c, NY93/c N-N^{pro} 18 EGFP (a mutant BVDV virus with the enhanced green fluorescent protein (EGFP) inserted between N^{pro} codons 18 and 19) and BRSV 236-652 viruses were used in the study. In vitro growth kinetics in single- and co-infected cells was evaluated using virus titration and real-time RT-PCR. An IFN reporter gene assay was used to determine IFN levels in cells following single- and co-infections. Results: NY93/c N-N^{pro} 18 EGFP replicated 0.4 - 1.6 TCID₅₀ logs lower than NY93-wt in Madin-Darby bovine kidney (MDBK) cells. MDBK cells infected with NY93/c N-N^{pro} 18 EGFP synthesized significantly greater levels of IFN-I than NY93/c and NY93-wt-infected MDBK cells. BRSV RNA levels were significantly greater in NY93-wt/BRSV co-infected bovine turbinatae (BT) cells than in NY93/c N-N^{pro} 18 EGFP/BRSV co-infected BT cells. BVDV RNA levels were lower in NY93/c N-N^{pro} 18 EGFP-infected BT cells than in NY93-wt-infected BT cells. NY93/c N-N^{pro} 18 EGFP single- and co-infected BT cells synthesized significantly higher IFN-I than NY93-wt single- and co-infected BT cells. Conclusions: These findings suggest: (1) NY93/c N-

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N^{pro} 18 EGFP BVDV2 induced higher levels of IFN-I than BVDV2-wt and may be useful as a safer, replicating BVDV vaccine, and (2) Enhancement of BRSV infection by BVDV co-infection is mediated by N^{pro} antagonism of IFN-I.

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Antigenic relationships among avian paramyxovirus serotypes

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Purpose: Avian paramyxoviruses (APMVs) have been classified into nine serotypes based on hemagglutination inhibition (HI) and neuraminidase inhibition tests. Newcastle disease virus (NDV) constitutes APMV-1 and is the best characterized APMV. Very little is known about APMV-2 to -9. In this study, the antigenic relationships among the APMV serotypes were studied by cross-HI and cross-virus neutralization (VN) tests using sera raised by a primary infection. Furthermore, the ability of immunity to APMV-2 to -9 to protect chickens against virulent NDV challenge was also evaluated. Methods: Oculo-nasal infections of 2-week-old chickens were carried with each APMV serotype. Twenty one days post infection, sera were collected and the chickens were challenged with NDV strain Texas-GB through the oculonasal route. The chickens were observed daily for two weeks for clinical signs and mortality. Challenge virus replication was quantified in different organs harvested 4 days post challenge. Antigenic relationships between the serotypes were analyzed by cross-HI and cross-VN tests.

Results: Primary infection of chickens with APMV-1 (strain LaSota), APMV-3 and APMV-9 conferred complete protection against virulent NDV challenge. APMV-2, -7 and -8 provided partial protection, whereas APMV-4 and -5 did not confer any protection. Both cross-HI and cross-VN tests showed one-way antigenic relationships between certain serotypes that were drastically reduced by two-way antigenic analysis.

Conclusions: Two-way antigenic analysis by HI and VN tests using single infection sera is a better method for serotyping of APMVs. Chickens with APMV-3 and -9 can provide protection against virulent NDV infections.

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Inhibition of NF-kappa B activation and suppression of interferon-beta production by Nsp1 alpha subunit of PRRSV.

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Induction of type I interferon (IFN-A/B) is an early antiviral response of the host, and porcine reproductive and respiratory syndrome virus (PRRSV) has been reported to downregulate the IFN response during infection in cells and pigs. We report that the PRRSV nonstructural protein 1A (Nsp1A) subunit of Nsp1 is a nuclear-cytoplasmic protein distributed to the nucleus and contains a strong suppressive activity for IFN-B production that is mediated through the retinoic acid-inducible gene I (RIG-I) signaling pathway. Nsp1A suppressed the activation of nuclear factor (NF)-kB when stimulated with dsRNA or tumor necrosis factor (TNF)-A, and NF-kB suppression was RIG-I dependent. The suppression of NF-kB activation was associated with the poor production of IFN-B during PRRSV infection. The C-terminal 14 amino acids of the Nsp1A subunit were critical in maintaining immunosuppressive activity of Nsp1A for both IFN-B and NF-kB, suggesting that the newly identified zinc finger configuration comprising of Met180 may be crucial for inhibitory activities. Nsp1A inhibited IkB phosphorylation and as a consequence NF-kB translocation to the nucleus was blocked, leading to the inhibition of NF-kB stimulated gene expression. Our results suggest that PRRSV Nsp1A is a multifunctional nuclear protein participating in the modulation of the host IFN system.

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Porcine reproductive and respiratory syndrome virus-associated glycans are potential targets for virus neutralization.

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Porcine reproductive and respiratory syndrome is the most severe infectious disease facing the swine industry worldwide. PRRSV has strict cell tropism, growing preferentially *in vivo* in porcine macrophages. *In vitro*, PRRSV grows in primary cultures of porcine alveolar macrophages, as well as in MARC-145 cells, which are routinely used for *in vitro* propagation of wild-type and vaccine strains of PRRSV. However, between the two types of host cells, a PRRSV strain usually has a growth preference on one cell type over the other. Understanding the molecular structure of PRRSV is expected to facilitate the study of cell tropism and the development of disease prevention strategies. PRRSV has a smooth spherical envelope embedded with glycoproteins. The broadly distributed viral glycans are likely to cover the virion surface, or even stretch out as antennae, thus may serve as potential targets for virucidal recognition resulting in virus neutralization. Herein, lectins that specifically bind glycans were screened for the ability to inhibit PRRSV infection. We found that in the same MOI condition, lectins specific for terminal mannose/glucose, terminal galactose and sialic acids inhibit viral infection similarly on the two cell types; whereas lectins specific for N-acetylglucosamine (GlcNAc) and N-acetylgalactosamine (GalNAc) inhibit viral infection to varying degrees depending on the host cell type. The virus-binding level of lectins was assessed by co-precipitation assays. Furthermore, we demonstrated that lectins inhibit infection by blocking the viral attachment to host cells. Finally, PRRSV was purified and mass spectrometry (LC-MS/MS) was applied to study the potential glycan compositions on viral GP5. Potential glycan compositions were identified and consistent with the lectin inhibition results. In conclusion, PRRSV-associated glycans are highly accessible for specific binding that can result in significant inhibition of viral infection, thus are potential targets for virus neutralization.

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Broader cross neutralization against PRRS viruses conferred by chimeric viruses containing structural gene fragments from distinct heterologous strains.

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Porcine reproductive and respiratory syndrome virus (PRRSV) remains to be one of most economically significant pathogens in the swine industry throughout the world. Vaccines are available and have played an important role in PRRS control. However, due to the high mutation rate of the virus, the protection provided by a monovalent vaccine is commonly limited to homologous virus strains; prevention of PRRS by vaccination is suboptimal. Thus, vaccines that can confer broad protection against not only homologous but also heterologous strains are highly desired. Our lab previously demonstrated that a VR2332-based chimeric virus possessing ORFs 2-4 from the VR2332 strain and ORFs 5-6 from the JA142 strain produced neutralizing antibodies that were equally effective on both donor strains and was neutralized by antisera raised against each of the donor strains *in vitro*. A subsequent *in vivo* study revealed that pigs vaccinated with the chimeric virus were protected from infection by both donor viruses. To further validate this strategy, 12

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additional chimeric viruses randomly combining ORFs 3-4 and ORFs 5-6 of four antigenically and genetically distinct PRRSV strains (1648-02, 17198-2, MN184 and SDSU73) were constructed using the VR2332-based infectious clone. Antisera against each donor virus were generated in 3-week-old pigs which were euthanized at 45 days post inoculation. Neutralizing activity of each serum against each of the chimeric viruses was tested *in vitro* by FFN assay. While the donor viruses could hardly be neutralized by heterologous antisera, the chimeric viruses were neutralized by antisera against donor viruses as effectively as were the homologous donor viruses. This observation suggests that the strategy of constructing chimeric viruses by combining the structural gene from distinct heterologous strains in an organized manner can provide broad protection and can be widely applicable as a novel vaccine platform for PRRSV.

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Genetic variation of PRRSV ORF5 following modified live PRRSV vaccination in a PRRSV positive herd.

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The objective was to investigate the genetic variation of PRRSV ORF5 following modified live PRRSV vaccine (MLV) use. A PRRSV positive stable swine herd with co-existence of EU and US genotypes and no history of MLV use was recruited. Following an outbreak, MLV (Boehringer Ingelheim, US genotype) was implemented. All sows were mass-vaccinated monthly for 2 consecutive months followed by every 3 months. All piglets were vaccinated at 7 days of age. Serum samples were collected monthly for six consecutive months from 4 population groups of 5 samples each including replacement gilts, sows, nursery and finishing pigs. ORF5 was amplified and clone into vectors for subsequent transformation. Five bacterial transformant colonies were randomly selected from each sample for purification and sequencing. The analysis was performed on isolates at each time point. Phylogenetic tree was constructed and percentage of nucleotide identity was determined. Prior to and following vaccination, both US and EU genotypes were isolated and co-existed in an individual pig. Following the vaccination, both genotypes exhibited similar manner as isolates of each genotype were clustered into 2 groups (A and B), approximately 11.1 and 13.6% nucleotide different from the predecessor. The nucleotide difference between 2 groups was 5 - 15%. Interestingly, the divergence of group A increased by time while that of group B remained the same. The results of the study suggested mutation increase by time. It remains to be further investigated whether MLV play a role in increased mutation.

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Development of Transgenic Mouse Model Susceptible to Porcine Epidemic Diarrhea Virus.

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Porcine epidemic diarrhea virus (PEDV) is a group I coronavirus and is specific to piglets. However, the use of pigs as the animal model has several disadvantages, relatively expensive, and large size. So, experimental animal models for PEDV infection are needed to study PEDV pathogenesis. We developed transgenic mice expressing porcine aminopeptidase N (pAPN, CD13), a functional receptor for PEDV, under the regulation of a proximal promoter. Transgenic mice clinically showed a mild weight loss and slight temperature increase after oral infection of PEDV. Virus presence was confirmed by RT-PCR only for tissue extracts from transgenic mice. Mice were killed at 1, 2, 3, and 5 days post inoculation and harvested intestines, lung, liver, spleen, kidney for pathologic examination. Transgenic mice were properly expressed pAPN and allow the study for PEDV infection, in particular, viral tropism and spread *in vivo*. Furthermore, these mice might be another tool for the isolation of wild type PEDV.

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Genetic and antigenic characterization of H1 influenza viruses from United States swine prior to the emergence of the 2009 pandemic H1N1.

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Swine play a role for the evolution of influenza A viruses. Prior to the introduction of the 2009 pandemic H1N1 virus from humans into pigs, four phylogenetic clusters of the hemagglutinin (HA) gene from H1 influenza viruses could be found in U.S. swine. Viruses from the classical H1N1 swine lineage evolved to form α -, β -, and γ -clusters whereas viruses with HA genes most similar to human seasonal H1 viruses emerged in 2003 to form the δ -cluster. Limited sequence information was available regarding the six genes that make up the triple reassortant internal gene (TRIG) cassette in contemporary H1 influenza viruses of swine. In addition, information regarding the antigenic relatedness of the H1 viruses was lacking due to the dynamic and variable nature of swine lineage H1. We characterized twelve H1 isolates from 2008 by sequencing and phylogenetic analysis of all eight gene segments and by serologic cross-reactivity in the hemagglutination inhibition (HI) assay. Based on genetic analysis, each of the four previously described phylogenetic clusters of H1 influenza viruses of swine were represented in the 2008 panel. Additionally, it was demonstrated that the δ -cluster HA were sub-divided into sub-clusters $\delta 1$ and $\delta 2$. Genetic diversity was demonstrated in all gene segments, but most notably in the HA gene. The genetic evolution of the NA gene was comparable to that of the HA gene. The gene segments from the 2009 pandemic H1N1 formed clusters separate from North American swine lineage viruses, suggesting progenitors of the pandemic virus were not present in U.S. pigs immediately prior to 2009. Serologic cross-reactivity paired with antigenic cartography demonstrated that the viruses in the different phylogenetic clusters are also antigenically divergent. Increased surveillance and monitoring of enzootic influenza viruses of swine as well as the 2009 pandemic H1N1 in the swine population worldwide are critical to understand the dynamic ecology of influenza A viruses in this susceptible host population.

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Viral reassortment and transmission after coinfection of pigs with Northern American triple reassortant and Eurasian H1N1 swine influenza viruses.

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Purpose: The 2009 pandemic H1N1 virus (pH1N1) is a reassortant virus between North American triple reassortant swine influenza viruses and Eurasian swine influenza viruses, which has caused the death of over 18,449 persons worldwide as of August 1, 2010. The genome of the pH1N1 contains HA, PB1, PB2, PA, NP and NS genes from currently circulating North American triple reassortant swine influenza viruses and NA and M genes from Eurasian swine influenza viruses. The pH1N1 has been transmitted from humans to other species including pigs, ferrets, turkeys, dogs and cats. To date, how, when and where the pH1N1 was generated is not well understood.

Methods: We dually infected pigs with North American triple reassortant swine H1N1 A/Swine/Kansas/77778/2007 (KS07) virus and Eurasian swine H1N1

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A/Swine/Spain/53207/2004 (SP04) virus, then co-housed with a group of sentinel animals. This direct contact group was subsequently moved into contact with a second group of naïve animals. Each individual gene segment of isolated viruses from co-infected and contact pigs can be identified using a unique restriction enzyme site and sequencing.

Results: Only three viruses were identified in bronchioalveolar lavage fluid collected from lungs of experimentally co-infected pigs: KS07, KS07 + SP04-NA and KS07 + SP04-NA-NS. The KS07 and KS07 + SP04-NA viruses were transmitted from the co-infected pigs to two contact groups of animals. However, only the intact KS07 virus was detected from nasal swabs collected from the second contact group pigs. These results demonstrate that multiple reassortant events can occur within the lower respiratory tract of the pig; however, only a specific gene constellation is able to be maintained and shed from the upper respiratory tract.

Conclusions: In conclusion, we were not able to generate the pH1N1 virus by co-infection of pigs with a currently circulating North American triple reassortant H1N1 virus and a Eurasian avian-like swine influenza virus. It seems that the triple reassortant KS07-like H1N1 viruses replicate very efficiently in pigs and out compete other viruses with different genetic constellations.

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Influenza Infection in Cats.

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Although cats have several features that would make them ideal vehicles for interspecies transmission of influenza viruses they have been overlooked as a potential reservoir and possible species for viral mixing. Studies suggest that cats may be infected with at least the H5N1 subtype of influenza by either respiratory or oral exposure and that they may shed the virus by both respiratory and fecal excretion providing diverse routes for transmission and contamination of the environment cohabitated by humans and other susceptible species. Recent incidences of pandemic H1N1 infection in cats further highlight the potential role of cats as intermediate or bridging host. This study was conducted to identify the range of influenza subtypes that can infect domestic cats. In addition, sero-surveillance was conducted to understand the prevalence of influenza infection in cats. In vitro infection and replication study using primary feline tracheal/bronchial epithelial cells showed that seasonal human H1 and H3 subtypes replicated more efficiently compared to swine H1 and H3 viruses. For low pathogenicity avian influenza viruses, H5N1 and H7N2 subtype viruses replicated better than H2N2 and H6N2 subtype viruses. This in vitro observation correlated with in vivo experimental infection study results where human H1N1, avian H5N1 and H7N2 influenza viruses replicated better than other strains tested after intra-tracheal inoculation into cats. The results also showed that avian H5N1 and H7N2 viruses replicated preferentially in the lower respiratory tract. However, none of the infected cats developed clinical signs. Serological surveillance for type A influenza antibody in 296 serum samples, collected from domestic cats, showed that 7.4% were positive and 8.4% of samples were suspect-positive. In conclusion, our findings indicate that currently circulating influenza viruses of human and avian origin are capable for replication in cats, and detection of type A influenza antibody in cats further support the potential role of cats for interspecies transmission of influenza virus.

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Comparative pathogenicity of novel avian-origin canine influenza virus H3N2 in various species.

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Novel avian-origin canine influenza A viruses (H3N2) were isolated and its pathogenicity and repeated infection in dogs identified in Korea. HA and NA genes of these isolates had greatest identity with those of Korean non-pathogenic avian influenza virus isolate, S11. In this study, the novel avian-origin influenza A virus (H3N2) was experimentally inoculated into several species including chicken, pigs, mice and ferrets, and compared its pathogenic effects in each species. Further more, pathogenicity of Korean avian influenza virus (S11 strain) in conventional beagle dogs was investigated. The novel canine influenza virus (H3N2) did not show any clinical signs, gross and histopathological lesions in pulmonary tissues of SPF chicken and pigs, however, it displayed a necrotizing bronchitis and interstitial pneumonia with neutrophil, alveolar macrophage and lymphocyte infiltration in mice and moderate to severe interstitial pneumonia in ferrets. Viral shedding and sero-conversion after inoculation were also detected in both species. Beagle dogs infected with S11 strain did not show any clinical signs, gross and histopathological lesions, and sero-conversion.

In summary, the novel canine influenza virus H3N2 had no pathogenicity in chicken and pigs, but in mice and ferrets even though its origin from bird, and avian influenza virus similar to the canine influenza virus did not show any evidence of infection to dogs. These results might indicate that the novel canine influenza virus H3N2 had virulence and infectivity in several mammals such as not only dogs but mice and ferret.

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Effective small interfering RNA cocktails targeting viral and avian genes as an alternative to vaccination for avian influenza.

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Purpose: Avian influenza (AI) is a high consequence, economically relevant disease of poultry. Disease behavior will influence viral spread patterns, diffusion ranges, amplification, persistence, and the likelihood for emerging high impact strains. The lack of vaccine that confers complete immunity and the pressures disease behavior could place on promoting the spread of virulent strains, underlines the urgency to accelerate the discovery and development of more effective control methods for AI in poultry. The goal of this study is to use RNA interference to develop a unique antiviral application for AI in an avian tissue model using small interfering RNAs (siRNAs). To our knowledge, there are no published reports indicating research targeting avian host genes with siRNA technology or the use of multi-siRNA cocktails has been considered. Our approach investigates siRNAs targeting viral and chicken genes both individually and in combination and is novel in terms of providing a more comprehensive prevention strategy so optimal effectiveness in prevention of AI is achievable.

Methods: The AI genes chosen for siRNA knockdown were the NP, PA, PB1, and PB2 segments. Chicken hepatocellular carcinoma epithelial (LMH) cells were transfected with each viral siRNA and infected with the H8N4 virus. In addition to mono-siRNA (individual) transfection, a combined viral siRNA cocktail was optimized. Using specific selection criteria, siRNAs were designed against nine chicken genes, but have yet to be evaluated. Assessment of AI infection, replication and the production of infectious viral particles were determined via immunocytochemistry staining for the AI NP antigen, RT-qPCR based on the AI matrix gene, and TCID50.

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Results: Individual and combinatorial siRNA mediated knockdown of the specified viral genes, followed by infection with the H8N4 virus has the potential to inhibit viral replication in vitro.

Conclusions: Future work will evaluate the 9 chicken gene siRNAs to determine if siRNA gene knockdown at the host level has the potential to inhibit AI replication. Targeting these viral and host genes could constitute an alternative and novel approach for future potent antiviral use in poultry.

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Viral induced inflammatory cytokines, toll like receptors and cytotoxic T cells components in IBDV- infected chickens.

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Infectious bursal disease (IBD) is an important immunosuppressive disease of chickens. IBDV consists of two serotypes 1 and 2. Serotype 1 consists of classic IBDV (cIBDV) and variant IBDV (vIBDV). Both of these strains vary in their pathogenesis. This study was conducted with two broad objectives: (1) to evaluate the differential expression of virus-induced innate and proinflammatory cytokines, chemokines and toll like receptors (TLRs) and their adaptor molecules; (2) to determine the role of cytotoxic T-cells in the pathogenesis of IBDV infection. To achieve the first objective, we inoculated 3-week-old SPF chickens with either cIBDV or vIBDV. At post inoculation days (PID) 3, 5 and 7, gene expression was determined in the bursa by quantitative RT-PCR (qRT-PCR). There was upregulation of innate cytokines (IFN α and IFN β), proinflammatory cytokines (IL-6 and IL-1 β), chemokines (IL-8 and MIP- α) and iNOS in cIBDV- and vIBDV-infected bursa. The expression of TLR3 was down-regulated at PIDs 3, 5, and 7 in the bursa of vIBDV-infected chickens whereas TLR3 was upregulated at PIDs 3 and 5 but was downregulated at PID 7 in cIBDV-infected chickens. In vIBDV-infected bursa, TLR7 expression was downregulated at PIDs 3 and 5 but upregulated at PID 7. However, TLR7 was upregulated at PIDs 3 and 7 in cIBDV-infected bursa. The expression of MyD88 was downregulated and TRIF gene expression was upregulated in cIBDV- and vIBDV-infected bursa. These findings indicate that cIBDV and vIBDV mediated the differential induction of proinflammatory cytokines and chemokines and regulated the expression of TLRs as well as their adaptor molecules. In the second half of the study, we evaluated the role of cytotoxic (CD8⁺) T-cells in the pathogenesis of IBDV. Infection of chickens with cIBDV was accompanied by the infiltration of CD8⁺ T-cells in the bursa. We also noticed the upregulation of gene expression of important cytotoxic molecules; perforin and granzyme A and their adaptor molecules HMG and PARP in the bursa whereas expression of NK lysine was downregulated. The findings of this study will help in the elucidation of mechanisms of IBDV pathogenesis and the role of cytotoxic T-cells in the clearance of virus-infected cells.

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Molecular characterization of fowl adenovirus fiber genes.

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Fowl adenoviruses (FAdVs) belong to the family *Adenoviridae*, genus *Aviadenovirus* that comprises 5 species of FAdVs and one goose adenovirus species. Inclusion body hepatitis (IBH) associated with infection by fowl adenoviruses affects mostly 3 to 7 weeks old meat-type chickens. FAdVs are endemic worldwide and can be isolated from both healthy and sick birds. The objective of this study was to analyze the nucleotide and deduced amino acid sequences of the fiber gene of four FAdV serotype 8 (FAdV-8) and four serotype 11 (FAdV-11) isolates and compare them to those of avian and mammalian adenovirus sequences. Two isolates in each serotype group were isolated from flocks with no clinical signs of IBH, while another two were isolated from flocks with clinical signs of IBH, and IBH was confirmed as the final diagnosis. The coding sequences of the fiber genes were 1572 and 1573 nucleotides (nts) long independent of serotype, encoding a polypeptide of 524 or 525 amino acids (aa). Like all adenovirus fiber proteins, the FAdV fibers could be divided into three regions, tail, shaft and head. The FAdV tail regions were 63 aa long containing poly(G) stretch which occurs in the tail of the long fiber of FAdV-1. The VYPY sequence in the fiber tail is a conserved sequence motif and found in all mammalian adenovirus fiber proteins. The equivalent sequence in the FAdV fiber genes was "YYPF" also reported in FAdV-1. Most mammalian adenovirus fiber sequences contain the 2-KR Δ R nuclear localization motif. A similar motif (RKRK) was also present in all analyzed FAdVs but at position 16 in the peptide chain. In the fiber shaft 16 pseudorepeats were recognized in all studied FAdVs, with each repetition comprising 18 aa on average. The knobs of the FAdVs did not have the conserved TLWT motif present in the mastadenovirus fiber protein, but a unique GMSS motif was present only in FAdVs. In order to evaluate the influence of adenoviral fiber gene variants on pathogenicity, experimental infection with different FAdV isolates would be necessary.

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New and emerging diseases: A very personal perspective.

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In recent years, emergent human disease episodes have increased dramatically; nearly all have involved zoonotic or species-jumping infectious agents. Because there is no way to predict when or where the next important new zoonotic pathogen will emerge or what its ultimate importance might be, initial epidemiologic and laboratory investigations and rapid public health responses are particularly important. Having spent most of my career working at this place in what I call the "discovery-to-control continuum," and having developed strong opinions about the present state of our systems to respond to new threats, I have a hope that the next generation of public health scientists might do better, in international, national, local, and academic venues. In recent years I have developed a keen interest in the historic foundations of the public health sciences, especially medical and veterinary virology - I will use several episodes from the past as lessons for the future. Because emerging zoonotic diseases are distinct from endemic diseases, their prevention and control often requires unique strategies, in my view based more on fundamental research to find "weak-links" than on traditional surveillance / action approaches (in veterinary medicine, "the sanitary rifle"). Such strategies require that we rebuild a cadre of career-committed professionals with a holistic appreciation of all of the pertinent medical, veterinary, biologic and ecologic sciences. Some colleagues wish to organize this under the flag, "One Health" (or "One Medicine"), but there is still a question whether this flag will have "legs" in medical circles as it seems to in veterinary circles, and whether it will generate the kind of substantial funding base that the flag, "New and Emerging Infectious Diseases" has over the past 20 years. We shall see!

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Infection of PBMC with Neuropathogenic Equine Herpesvirus Type 1 Ab4 strain induces interferon-alpha and modulates interleukin-10 production.
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Purpose: Equine herpesvirus 1 (EHV-1) is highly prevalent in most horse populations. Horses are routinely vaccinated against EHV-1 and neutralizing antibodies have helped to prevent disease. However, EHV-1 myeloencephalopathy (EHV-1 - EHM) has recently been classified as an emerging disease by the USDA due to the apparent increase in incidence, morbidity, and mortality of neurological disease suggesting a change in virulence of the virus. It was reported that cellular immune mechanisms, in particular cytotoxic T-cells (CTLs), are important to control EHV-1 viremia. Interferon-alpha (IFN- α) has a key function in innate immune regulation by inducing the differentiation and maturation of CTLs. Here, we investigated the influence of abortogenic (RacL11, NY03) and neuropathogenic (Ab4) EHV-1 virus strains on IFN- α , IL-4 and IL-10 secretion in equine PBMC.

Methods: Equine PBMC were infected with RacL11, NY03 or Ab4 EHV-1 strains or kept in medium for 24 hours. IFN- α , IL-10 and IL-4 secretion was detected in the supernatants by a fluorescent bead-based cytokine assay.

Results: The production of IFN- α increased with increasing viral doses and similarly for all three EHV-1 strains. The production of the anti-inflammatory cytokine IL-10 was significantly decreased after Ab4 infection compared to RacL11 and NY03 strains at viral infection doses of MOI 0.3-1. At high doses (MOI 3), IL-10 production was suppressed by all three EHV-1 strains.

Conclusion: The results suggested that abortogenic and neuropathogenic EHV-1 strains equally induce anti-viral IFN- α production in equine PBMC. They also illustrated the differences in the ability of EHV-1 strains to modulate anti-inflammatory IL-10: Neuropathogenic Ab4 strain had an increased potential to down-regulate IL-10 production suggesting to specific viral mechanisms that interfere with the control of inflammation in the host. The variations in innate IL-10 secretion might influence the development of protective immunity and might offer an explanation why neuropathogenic Ab4 induces more severe disease, including myeloencephalopathy, than abortogenic EHV-1 strains.

This work was supported by the Harry M. Zweig Fund for Equine Research.

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Equine herpesvirus type-1 modulates CCL2, CCL3, CCL5, CXCL9, and CXCL10 chemokine expression.

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Purpose: Equine herpesvirus type 1 (EHV-1) is highly prevalent in horses and causes rhinopneumonitis, abortion, and encephalopathy. Studies on the related human herpes simplex virus and from murine models of EHV-1 suggest that chemokines play important roles in coordinating of innate and adaptive immune responses, and thus effective control of herpesvirus infection and prevention of severe clinical disease.

Methods: Equine peripheral blood mononuclear cells (PBMC) were infected with one of three EHV-1 strains, which differ in pathogenicity (RacL11, NY03 = abortogenic, Ab4 = neurogenic). Changes in CCL2, CCL3, CCL5, CXCL9 and CXCL10 chemokine gene expression relative to non-infected PBMC were measured by real-time PCR.

Results: CXCL9 and CXCL10 gene expression was up-regulated 10 hours post infection and decreased to the level of non-infected cells after 24 hours. CCL2 and CCL3 were significantly down-regulated 24 hours post infection with NY03 and Ab4. CCL5 was up-regulated 24 hours after infection with RacL11. Ab4 infected PBMC had significantly lower expression of all chemokines except CCL2 24 hours post infection than RacL11 infected cells. While there was not a significant difference between NY03 and the other strains, there was a trend with each chemokine toward NY03 inducing less expression than RacL11 but more than Ab4.

Conclusions: The data suggested that EHV-1 infection of PBMC induced up-regulation of inflammatory chemokines CCL5, CXCL9 and CXCL10, and down-regulation of chemotactic CCL2 and CCL3. The data also implies that different EHV-1 strains have varying effects on all five chemokines, with the neuropathogenic strain, Ab4, having the greatest suppressive potential.

This work was supported by the Harry M. Zweig Fund for Equine Research.

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