PROGRAM and PROCEEDINGS

of the 92nd Annual Meeting
December 4, 5 and 6, 2011

Marriott, Downtown Magnificent Mile
Chicago, Illinois

Robert P. Ellis, Executive Editor

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

The 92nd Annual Meeting of the CRWAD is dedicated to

Dr. Donald G. Simmons

Proceedings Distributed by CRWAD

CRWAD 92nd ANNUAL MEETING-2011

December 4 – 6, 2011

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 4, 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 6
Dedication of the 2011 meeting to Dr. Donald G. Simmons
Introduction of New Members and Graduate Student Awards Presentations

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

Speaker Ready Room is: Streeterville Room (2nd floor) - Sunday, Dec. 4 - Monday, Dec. 5

Marriott Hotel Section	Monday AM 8:00 - 11:30 Room Abstract Nos.	Monday PM 1:30 - 4:30 Room Abstracts Nos.	Tuesday AM 8:00 - 11:30 Room Abstracts Nos.
Bacterial Pathogenesis	Avenue Ballroom 001 - 007	Avenue Ballroom 008 – 018	Avenue Ballroom 019 – 024
Biosafety and Biosecurity		Denver/Houston 025 – 030	
Companion Animal Epidemiology			Denver/Houston 031 – 039
Epidemiology and Animal Health Economics	Salons A/B/C/D 040 – 051	Salons A/B/C/D 052 – 062	Salons A/B/C/D 063– 071
Food and Environmental Safety	Salon E 072 – 083	Salon E 084 – 093	Salon E 094 – 100
Gastroenteric Diseases	Michigan/Michigan State 101 – 108		
Immunology	Salons F/G/H 109 – 120	Salons F/G/H 121 – 129	Salons F/G/H 130 – 136
Respiratory Diseases	Indiana/Iowa 137 – 148	Indiana/Iowa 149 – 157	
Vector-Borne and Parasitic Diseases	Denver/Houston 158 – 168		
Viral Pathogenesis	Los Angeles/Miami 169 – 178	Los Angeles/Miami 179 – 185	
Posters* in Grand Ballroom	Salon III-7th Floor Sun. 6:30 - 8 PM	Salon III-7th Floor Mon. 5 - 6:30 PM	

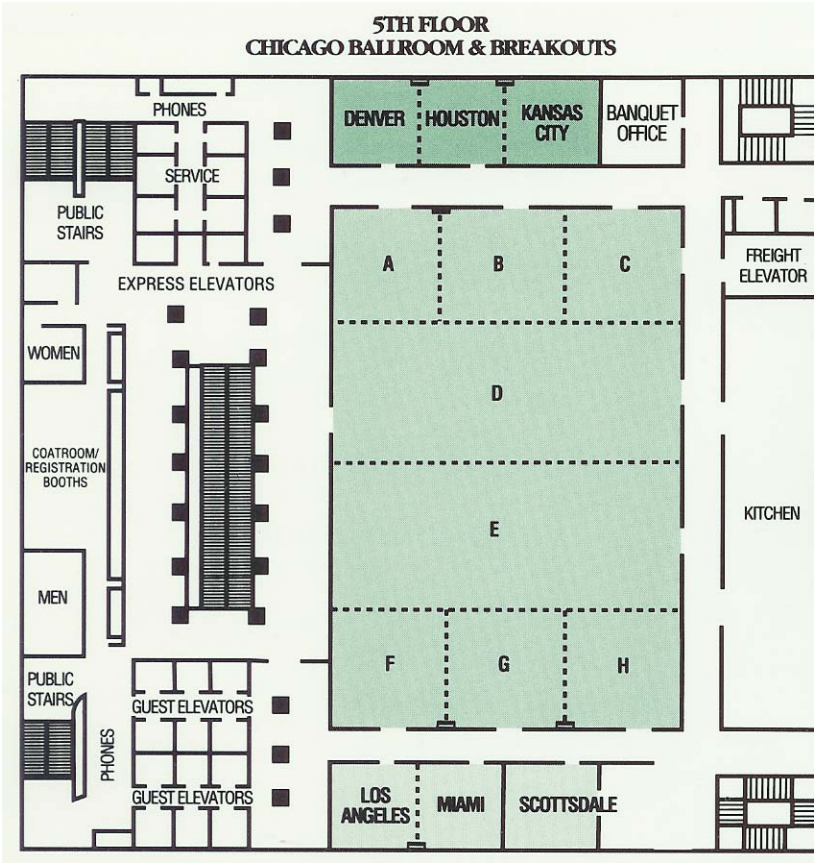
***SUNDAY POSTER PRESENTERS**: Poster boards will be available for poster assembly by 4 PM Sunday. Posters for the Bacterial Pathogenesis, Biosafety and Biosecurity, Companion Animal Epidemiology, Epidemiology and Animal Health Economics, Food and Environmental Safety, and Gastroenteric Diseases Sections 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.

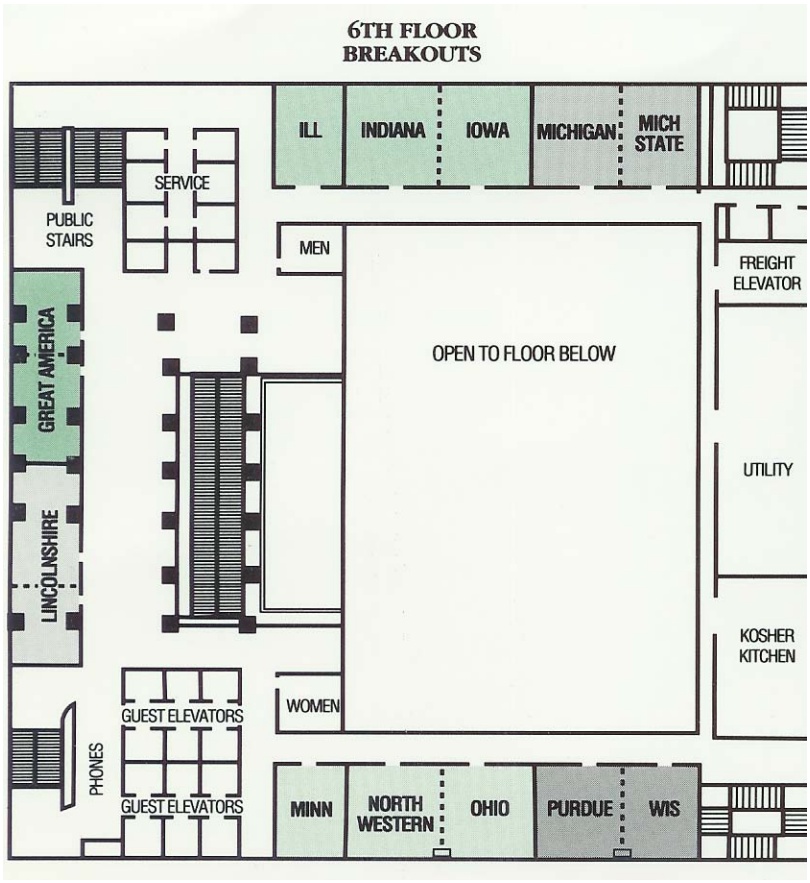
***MONDAY POSTER PRESENTERS**: Poster boards will be available for poster assembly by noon Monday. Posters for the Immunology, Respiratory, Vector-Borne and Parasitic Diseases, and Virology Sections will be presented Monday from 5:00-6:30 PM. Please remove your posters immediately upon completion of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

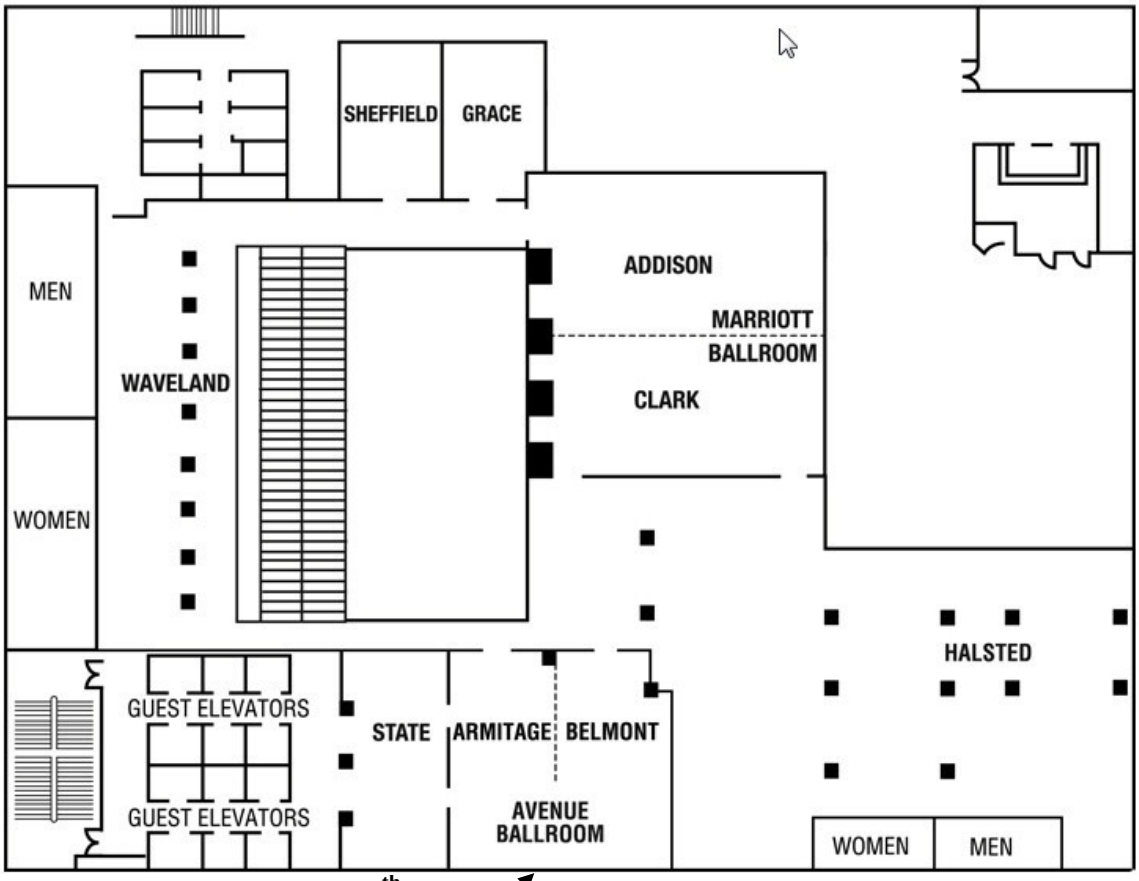
Poster Boards are 4 ft tall x 8 ft wide. Poster presenters must furnish their own tacks.

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

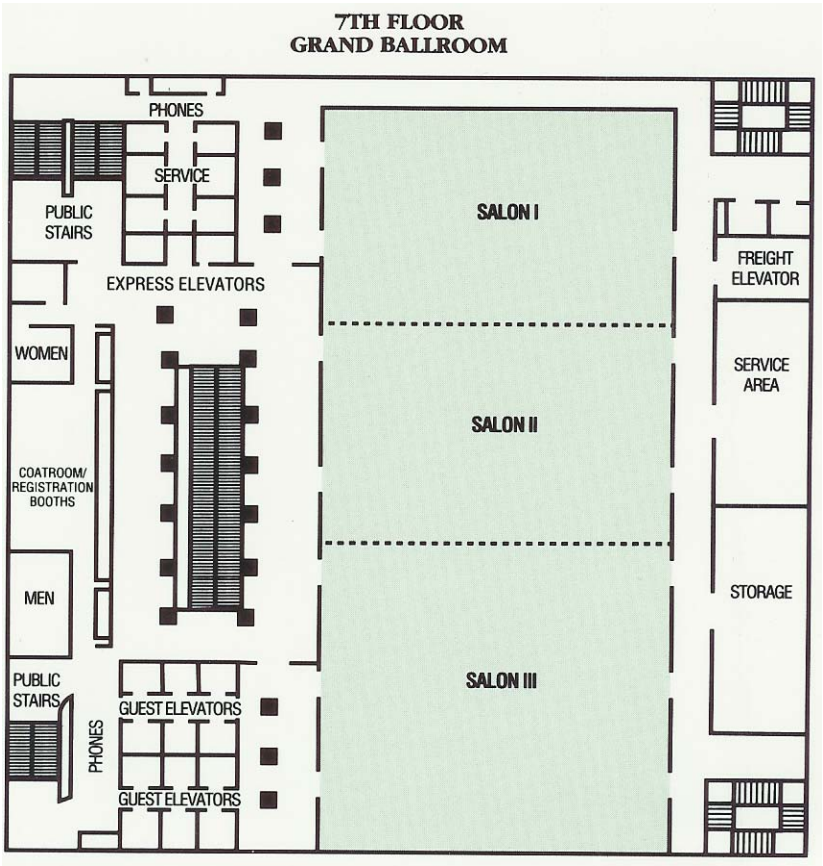




Chicago Marriott Floor Plan – 4th and 7th Floors



Avenue Ballroom on 4th Floor



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

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ABSTRACTS

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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2011 Officers

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Vice President - Donald L. Reynolds
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Patricia E. Shewen - 1996
Ronald D. Schultz - 1994
Richard F. Ross - 1992
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Bill Stich - 2009
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Ian Gardner - 2005
Katherine M. Kocan - 2003
Linda J. Saif - 2001
M. D. Salman - 1999
Bert E. Stromberg - 1997
Bradford B. Smith - 1995
Lawrence H. Arp - 1993
Robert M. Corwin - 1991
William C. Wagner - 1989

The Dedicatee Tradition

Each year, we select a Life member who has made outstanding contributions to CRWAD and to animal disease research to be honored as the Dedicatee for the CRWAD Annual Meeting. This tradition was initiated in 1974. Each Dedicatee is invited to attend the Annual Meeting as our guest. At the Business Meeting, the meeting is formally dedicated to the Dedicatee and the Dedicatee is given a plaque and an honorarium. Past Dedicatees and the 2011 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
Donald G. Simmons	2011	Samuel K. Maheswaran	2010

2011 CRWAD Dedicatee – Donald G. Simmons

A native of Virginia, Dr. Don Simmons earned a BA in Biology from Bridgewater College, Bridgewater, VA. After graduation he moved to Athens, Georgia where he completed a DVM, MS and PhD at the University of Georgia. Shortly thereafter, he became a Diplomate in the American College of Veterinary Microbiologists.

Dr. Simmons was employed by North Carolina State University (NCSU) Department of Poultry Science for many years where he conducted research on viral and bacterial diseases of turkeys. In the late '70s he moved to NC State's newly created Veterinary Science Department and served as one of the "founding fathers" for the NCSU College of Veterinary Medicine. For a number of years he taught Veterinary students and continued his research in turkey diseases with a strong emphasis on *Bordetella avium*. Dr. Simmons has published over 50 refereed scientific journal articles, presented scientific papers at many local, national and international meetings and served as advisor to a number of graduate students.

In 1988, Dr. Simmons became Head of the Department of Veterinary Science, College of Agriculture and Life Sciences, Penn State University, State College, PA. In that role he provided leadership for teaching, research and extension programs, and for the diagnostic laboratory operated by the department/college.

Dr. Simmons was later employed by the American Veterinary Medical Association (AVMA) for 11 years as Director of the Division of Education and Research. In that role he was responsible for accreditation of all U.S. (and some non-U.S.) veterinary colleges, accreditation of U.S veterinary technician teaching programs and certification of foreign Veterinary graduates. He also oversaw / facilitated the Council on Research, all the specialty organizations offering Diplomat status, and other AVMA programs including international relations.

Dr. Simmons retired in 2007 and returned to NC where he enjoys being a "gentleman farmer", gardening, reading and working in his shop. He also enjoys his family and traveling.

2011 CRWAD - Distinguished Veterinary Microbiologist

Y.M. "Mo" Saif
The Ohio State University, Wooster, OH

The recipient of the ACVM Distinguished Veterinary Microbiologist award for 2011 is Dr. Y.M. "Mo" Saif. Dr. Y.M. Saif is internationally renowned for his expertise in infectious diseases of domestic poultry. This is evidenced by the fact that he has served as major editor of the world's foremost textbook in poultry diseases entitled "Diseases of Poultry." This widely used textbook is the standard and universal reference text for infectious diseases of poultry. He is Editor-in Chief of the 12th edition (2008) and 11th edition (2003) and is co-editor of the 10th (1997) and the 9th edition (1991) of this book. Dr. Y.M. Saif has spent his research career of approximately 35 years in the elucidation of the diagnosis, pathogenesis, immune response and prevention and control of several economically important enteric, respiratory and immunosuppressive diseases of poultry including viral, fungal and bacterial diseases of poultry. He has authored or co-authored approximately 140 refereed publications, 16 book chapters, 120 published abstracts, 100 non-refereed publications, and has made numerous presentations as an invited guest speaker. He has been involved in international activities related to infectious diseases of poultry in several countries of Europe, the Middle East, Latin America and Asia.

Dr. Y.M. Saif is Professor and Head, Food Animal Health Research Program, Ohio Research Development Center and Assistant Dean, College of Veterinary Medicine, The Ohio State University. Through the years he has mentored several Ph.D. students who have been successful in their careers. He has advised approximately 45 Ph.D., M.Sc. students and postdoctoral fellows, and has served on the graduate committees of 49 Ph.D. and M.Sc. students. He has received many awards for his work such as Special Service Award (2006) from the American Association of Avian Pathologists, Excellence in Poultry Research Award (1996) from American Veterinary Medical Association, Graduate Education Research Award (1995), Distinguished Research Award (1991), Beecham Award for Research Excellence (1990) and many more as shown in his curriculum vitae.

Dr. Y.M. Saif has been very active in several veterinary professional organizations including the American College of Veterinary Microbiologists where he served as the Chair of the Board of Governors in 1982-1983 and Chair-Elect in 1981-1982. Additionally, he has served on several ACVM Committees over the years. He has also participated (as a committee member and/or chair) on several national, regional and university committees in many areas including food safety, agriculture and environmental sciences. He is a world renowned scientist and has made numerous contributions to our understanding of viruses. Through the years he has made tremendous contributions in veterinary medicine. Dr. Saif has been a long time member of the Conference of Research Workers in Animal Diseases (CRWAD).

2011 CRWAD - Keynote Speaker - Immunology Section
AAVI Distinguished Veterinary Immunologist Award

Dr. Patricia E. Shewen - Department of Vet. Pathobiology, University of Guelph, Guelph, Ontario, Canada

Abstract No. 121 - Title: Immunology and Animal Health: the whole is greater than the sum of its parts.

Pat Shewen is a graduate of the University of Guelph where she received B.Sc. and D.V.M. degrees, and following a few years of veterinary practice in Toronto, an M.Sc. in Clinical Studies and a Ph.D., as a Medical Research Council Fellow, in Immunology. She joined the faculty of Guelph's Ontario Veterinary College (OVC) in 1982, where she is now professor emerita. Her research has focused on immunity in infectious diseases of ruminants, in particular Chlamydial infertility in sheep and bovine pneumonic pasteurellosis, with recent emphasis on induction of immunity in neonates. This work has been funded by the Natural Sciences and Engineering Research Council of Canada, the Canadian Institutes for Health Research, the Ontario Ministry of Agriculture Food and Rural Affairs, as well as producer groups, including The Canadian, B.C. and Ontario Cattlemen's Associations, the Alberta Beef Producers, the Dairy Farmers of Canada and Ontario Sheep Producers. She is the author or co-author of over 100 research papers and book chapters, holds 5 patents, and has directly advised more than 30 graduate students and post-docs, who now hold prominent positions in governmental agencies, universities, and the animal health industry. Dr. Shewen considers the mentorship of graduate and undergraduate students a key aspect of her role as an academic. An invited speaker at several international and national scientific conferences, she also organized three international scientific meetings at Guelph, including the 1st International Veterinary Immunology symposium in 1986, with colleague Bruce Wilkie. Assistant Dean Research in 1996 and 1997, and inaugural Chair of the Department of Pathobiology 1997-2002, she was the first woman to hold senior administrative positions in OVC's 150 year history. More recently, Dr. Shewen completed two terms on the University's Board of Governors, and was appointed to the Governing Council of the National Research Council of Canada From 1997-2003. Pat has been a member of the American Association of Veterinary Immunologists since its inception and has served on the boards of both the AAVI and the Conference of Research Workers in Animal Diseases.

DAVID T. BERMAN – In Memoriam 1920 - 2010

Memorial Resolution of the faculty of the University of Wisconsin:

Professor Emeritus David T. Berman, Madison, WI, died July 8, 2010 after a long and distinguished career as a scholar, scientist, educator and academic administrator. He was born June 14, 1920 in Brooklyn, New York, the youngest of four sons of Russian-Jewish immigrant parents. The product of New York public schools, he set his professional course as a scientist at an early age as he explored the intellectual bounty of the Brooklyn Children's Museum, the American Museum of Natural History and the New York Public Library. In his University of Wisconsin Archives Oral History interview he said, "I just knew that the natural sciences were something for me and that biology was in particular what I wanted to do." He graduated from Brooklyn College, in 1939, with a major in biology. In the interview he indicated that while taking bacteriology courses, "I loved it and learned quickly that I wanted to do research." It was also at Brooklyn College where he met his future wife, Rhoda.

He intended to go to medical school but, confronted with strict quotas imposed by medical schools on the number of Jews who would be admitted and the lingering effects of the Depression on his family's financial resources, he attended the Cornell University College of Veterinary Medicine. At that time, that institution offered low tuition to qualified New York state residents. He graduated from Cornell with the degree Doctor of Veterinary Medicine in 1944. Following receipt of his DVM, he attended graduate school at the University of Wisconsin Madison and received his MS and PhD degrees in 1949 and 1950. He accepted a faculty position in 1951 in the Department of Veterinary Science at the University of Wisconsin and embarked on a distinguished career of teaching, research and administration until his retirement in 1987. He served as graduate advisor and mentor to many students from this country and abroad, all of whom share their lasting and profound respect for Professor Berman as a teacher, scientist and caring human being.

Professor Berman's tenure at the university included service as Chairman of the Department of Veterinary Science (1964-1968) and Associate Dean of the Graduate School (1969-1976), as well as a member of many departmental, college and campus committees. Among those committees and the time he served were: University Committee (1980-1983) serving as chair during 1982-83; Faculty Senator (1983-85) and Faculty Senate Alternate (1985-87); PROFS, Inc, Steering Committee (1980-84); Primate Center Committee (1969-1975); Graduate School Research Committee (1969-1975); Cancer Research Committee (1970-1975); Biotron Advisory Committee (1970-1975); Protection of Human Subjects Committee (1971-1974) serving as chair during1973-1974; Research Animal Resources Committee chair (1971-1975); Student Housing Advisory Committee (1971-1972); Graduate School Administrative Committee (1973-1975); Equity in Graduate Appointments Committee (1974-1977); Faculty Consultative Committee on Financial Emergency (1980-1982); and Commission on Faculty Compensation and Economic Benefits (1982-1983).

Professor Berman was a scientist of the highest order. He was the author/coauthor of more than 150 peer reviewed papers that focused on infections and immunity. Although his research and expertise encompassed many areas of infectious diseases, especially notable among them were his studies on brucellosis which led to the control and eradication of that disease of cattle and human beings in the United States. In 1976, at the request of the USDA, he chaired a national technical commission for a comprehensive study of the nationwide brucellosis eradication program. In 1980, the US Animal and Plant Health Inspection Service, the US Animal Health Association and the Association of State and Federal Veterinarians gave the Animal Health Award to Professor Berman, individually as commission chair, and to the technical commission as a body for their contributions to the brucellosis eradication program. Dr. Berman also received the Animal Health Award in 1990 for the success of his graduate study program for state and federal veterinarians.

Professor Berman served for many years on expert advisory committees for the World Health Organization and the Food and Agriculture Organization especially for their brucellosis programs. As part of that activity he went to several countries as an advisor on behalf of those organizations. In 1963 he took a study leave at the State Serum Institute in Denmark and at the Central Veterinary Laboratory, Weybridge, England.

In 2009, the 5th International Veterinary Vaccines and Diagnostics Conference, held in Madison, recognized him for his many contributions to research and graduate study in the veterinary medical sciences. Many of his former graduate students came from far away places to participate in that special event. The number who attended were testimony to the great respect for him as mentor and teacher, highly respected scientist and dear friend.

Dr. Berman is survived by his wife of 65 years, Rhoda, two children Morris (Joanne), Sara (Emily Sample), two grandchildren, Mateo and Hector, sister-in-law Mary Berman, and many cherished nephews, nieces, grandnephews and grandnieces.

Memorial Committee:

Charles Czuprynski
Bernard Easterday
Ronald Schultz, Chair

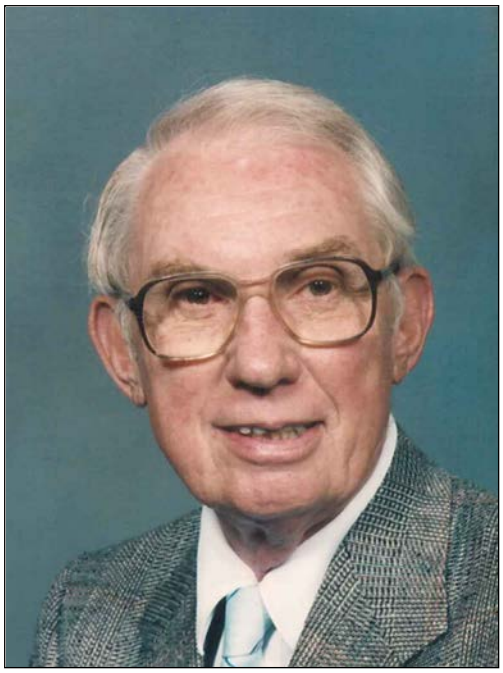
Dr. Berman was a member of the Conference of Research Workers in Animal Diseases and became a Life Member of CRWAD in 1993.

DR. EARL J. SPLITTER – In Memoriam - 2008

Earl J. Splitter (Kansas State University - 43). Dr. Splitter of Plantation, FL died Dec. 24, 2008. Following graduation he was a junior state Veterinarian with the North Carolina Department of Agriculture from 1943-1945. In 1946 he became an assistant, then associate professor at Kansas State University and received his Master of Science degree in 1950 and was an expert in haemoprotozoon diseases. He was noted for his work on eperythrozoonosis, anaplasmosis, and theileriosis. In 1957 he became Principal Veterinarian of Science Education Administration Cooperative Research of the USDA and later became the Acting Deputy Director. In this position, he was responsible for animal health research programs and special research grants in colleges of agriculture and schools of veterinary medicine in the United States. During his tenure, he served on the Science and Education budget team for animal protection; worked on animal health legislation, policy development, and congressional inquires. He was the veterinary medical coordinator of the US-USSR Agreement in Agricultural Research and Technology serving as the USDA negotiator in Moscow from 1977-1979 and as a host for Soviet veterinary teams as well as exchange programs with Spain and China.

Dr. Splitter was a member of numerous committees and organizations including the Conference of Research Workers in Animal Diseases, the AVMA, the National Association of Federal Veterinarians, and the American Association of Veterinary Parasitologists. He was awarded the American Feed Industry Association award in 1986 in recognition of his outstanding research contributions to livestock and poultry as well as the Conference of Research Workers in Animal Diseases dedicatee. In 1982 he was honored for distinguished service to Kansas State University College of Veterinary Medicine.

Dr. Splitter is survived by his wife, two daughters and one son who is a veterinarian (KSU 69) and is a faculty member of the University of Wisconsin-Madison.



DR. EDWARD HOMER BOHL
In Memoriam 1921 - 2011

Dr. Edward Homer Bohl, D.V.M., 89, of Wooster, OH, died Tuesday, July 19, 2011, at West View Manor Nursing Home in Wooster.

Edward was born October 16, 1921, in Georgetown, the son of Charles Henry and Nettie Agnes (Remley) Bohl, and graduated from Lebanon High School, (Lebanon, Ohio) in 1939. Edward graduated with a doctor of veterinary medicine degree in 1944 from Ohio State University.

He served with the U.S. Army in Europe during W.W. II, and married Jessie A. Pettigrew on September 21, 1947, in Corning, Ohio. They were married for 62 years. He then completed a master's degree in 1948 and his Ph.D. degree in 1952, all from Ohio State University.

Dr. Bohl was a world-renowned professor of veterinary microbiology at Ohio Agricultural Research and Development Center (OARDC) from 1963-82, and prior to that he was a professor at Ohio State University (OSU) College of Veterinary Medicine in Columbus. He had a large network of OSU and OARDC graduate students and colleagues.

He was a member of Wooster United Methodist Church, was past president of Wayne County Chapter of the Izaak Walton League and was a member of Wooster Kiwanis Club. Dr. Bohl was the 1995 Conference of Research Workers in Animal Diseases (CRWAD) honored dedicatee. He enjoyed spending time outdoors, fishing, gardening, playing tennis and visiting family and friends.

Surviving are sons, Phillip Bruce (Theresa) Bohl of Canton, Michigan, Edward Eric (Karen) Bohl of New Richmond, Wisconsin, and David Christian Bohl of Woodbridge, Virginia; grandchildren, Jennifer Bohl, Jason Bohl, Nancy (Matthew) Bohl Bormann, Katie (Jeffrey) Osterhaus, Charles (Maria) Bohl, Edward S. Bohl and Kristine Bohl; a great-grandson, Reese Bormann; and many nieces and nephews.

Edward was preceded in death by his wife, Jessie; his parents; and siblings, Walter Bohl, Howard Bohl, Richard Bohl, Mary Glover and Charles Bohl.

DR. GORDON R. CARTER – In Memoriam 1918 - 2011

Dr. Gordon R. Carter passed away on July 12, 2011 at age 93 in Blacksburg, Virginia. At the time of his death he was a Professor Emeritus of the Virginia-Maryland Regional College of Veterinary Medicine at Virginia Tech having retired in 1986.

Gordon, or Nick as he was known to friends, was born in Brandon, Manitoba, Canada on March 17, 1918. He often spoke fondly of his early years in Rockwood, Ontario, Canada. He received much of his education in Ontario, completing his DVM at the Ontario Veterinary College in 1943. At Iowa State University, Gordon completed his MS and at the University of Toronto a DVSc (Degree in veterinary microbiology).

He began work during WWII at Connaught Laboratories, where he met his future wife Martha, developing vaccines for the allied troops. Other positions included two years as Veterinary Officer for the Jamaican government and several years working for the Canadian government as a researcher on foreign animal diseases at Grosse Ile, Quebec. Between 1958 and 1962, Gordon worked for the Columbo Plan in Rangoon, Burma and the Food and Agriculture Organization of the United Nations in Bangkok, Thailand.

In 1963, Gordon continued his career in the United States as director of the Clinical Microbiology Lab at Michigan State University for over 15 years, where he received the Distinguished Faculty Award in 1978 and continued research on Pasteurella and other animal pathogens. In 1981, he joined the initial faculty of the Virginia-Maryland Regional College of Veterinary Medicine. While at MSU and Virginia Tech, Gordon served as a consultant on animal diseases for several international agencies in Southeast Asia, Brazil, and Africa. His field guide, "Diagnostic Procedures in Veterinary Bacteriology and Mycology" has been translated into numerous languages and is used extensively in the developing world.

He is best known for the diverse group of graduate students he advised, his identification of the capsular serogroups of Pasteurella multocida, publication of more than 100 scientific papers, and the authorship of many books, two of which went through five editions in veterinary microbiology.

Dr. Carter was the 1997 Conference of Research Workers in Animal Diseases (CRWAD) honored dedicatee. In 2007, he was the recipient of the Distinguished Veterinary Microbiologist Award given by the American College of Veterinary Microbiologists.

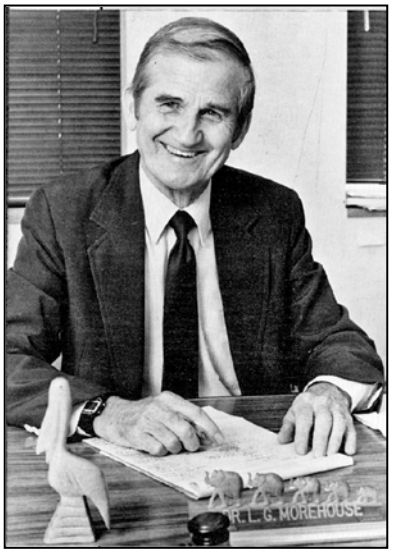
Gordon is survived by his wife of 61 years Martha Jane (Eady) Carter of Blacksburg, Virginia. He is also survived by his daughter Marion Carter, (Harry) Hoe of Ewing, Virginia and son Nicholas P. Carter of Blacksburg, VA. He has three grandchildren, all of whom admire him greatly: Lucas Carter Hoe, Miami, FL, Benjamin Carter Hoe, Pittsburgh, PA, and Marina Carter Hoe, Lexington, KY. He also leaves behind special friend Loretta Alls of Blacksburg, VA.

Gordon was a man of tremendous intellect and wit with a relentless curiosity and love of books, which he maintained until his death. He inspired many in his field and will be greatly missed by his family and associates around the world.

DR. LAWRENCE G. MOREHOUSE – In Memoriam 1925 - 2011

As note about Dr. Larry Morehouse from Dr. Bill Fales:

Larry arrived at the College of Veterinary Medicine in 1964 as Chairman of the Department of Veterinary Pathology. He was responsible for developing the Veterinary Medical Diagnostic Laboratory in 1968 and served as the Director from 1968 to 1987 and then as Interim Director until 1988 while a search was conducted for the Directorship. All of us who knew him and worked with him are saddened by his passing. His obituary is as follows:



Dr. Lawrence G. Morehouse, professor emeritus of veterinary pathology and retired director of the veterinary diagnostic laboratory at the University of Missouri, died of congestive heart failure on Wednesday, March 2, 2011, at Boone Hospital Center. He was 85.

Dr. Morehouse was born on a farm near Manchester, Kansas, on July 21, 1925. He graduated from a one room schoolhouse and Abilene High School. He served in the Pacific as a pharmacist's mate during World War II before returning to Kansas State University where he earned his BS and DVM in 1952. After a year of practice in the St. Louis area, he went to Purdue University. While he was working on his MS and PhD, he also served as the director of the USDA's brucellosis laboratory. He married Georgia Lewis in Lafayette, Ind., on October 6, 1956.

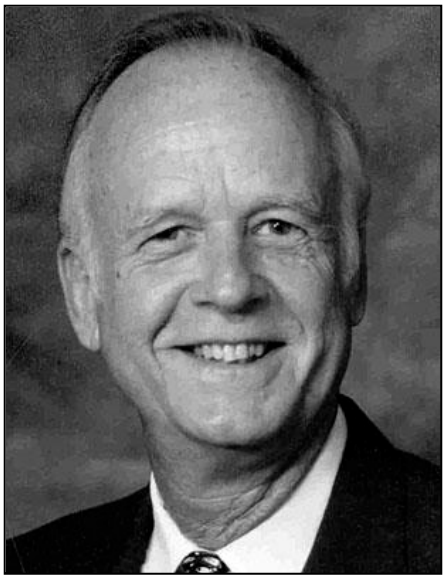
From 1960 until 1963, he worked for the USDA out of Washington, DC and later, at the newly established National Animal Disease Laboratory in Ames, Iowa. In 1964, he accepted a position as professor and chairman of the department of veterinary pathology at the University of Missouri. In 1968, he became the organizing director of the Veterinary Medical Diagnostic Laboratory and served in that capacity until he was named professor emeritus in 1987. During his career Dr. Morehouse authored or co-authored more than 100 scientific publications dealing with diseases of livestock, laboratory and companion animals. He was co-editor of a three volume encyclopedic work on fungal toxins. He was a member of a large number of professional organizations including the American and Missouri Veterinary Medical Associations, The American Association of Veterinary Laboratory Diagnosticians where he served as president and was recipient of its prestigious E.P. Pope award, The World Association of Veterinary Laboratory Diagnosticians (secretary/ treasurer), the U. S. Animal Health Association, Sigma Xi, The Royal Society of Health, The Conference of Research Workers in Animal Diseases, and many more.

In his retirement, his interest in genealogy led to his publishing a Morehouse Family genealogy. At the urging of his wife and children, he wrote an autobiography entitled "The Nine Lives of L. G. Morehouse".

He was a member of the M. Graham Clark Chapter of the Sons of the American Revolution as well as the Mayflower Society. He also served as president of the MU Retiree's Association. Dr. Morehouse continued to attend the CRWAD meetings after he became a CRWAD Life Member.

Dr. Morehouse was a long time member of Trinity Presbyterian Church, Columbia, where he served several terms as elder and also as clerk of session. He was passionate about his love of music, especially opera and served on the board of the Missouri Symphony Society.

He is survived by his wife, Georgia, a son, Timothy Morehouse, his wife Kara and their sons Gabriel and Elijah, of New Jersey, and a daughter, Glenn Morehouse Olson, her husband Joel and their daughters, Zoey, Abilene and Jolie.



DR. PHILLIP A. O'BERRY – In Memoriam
1933 – 2011 **(**Feb. 1, 1933 - July 28, 2011)

Phil O'Berry has spent the past few weeks embracing friends and family while telling them how much they have meant to him. Phil died of colon cancer on July 28, 2011. His miraculous survival for over seven years has been inspirational to those privileged to watch him live life to the fullest. His message for all the friends he left behind is, "Get a regular colonoscopy starting at age 50. Colon cancer IS preventable!"

Phil was born Feb. 1, 1933, in Tampa, Fla. He attended Miami Senior High and graduated in animal husbandry from the University of Florida in 1955. After earning a degree in veterinary medicine from Auburn University in 1960, he received a Ph.D. in veterinary microbiology from Iowa State University in 1967.

Phil married Terri Martin O'Berry in Miami on July 31, 1960. They spent a year in the Washington, D.C. area and then moved to Ames for Phil to work at the National Animal Disease Center as he pursued his Ph.D. Phil and Terri have six children, Kelly (Kasia) O'Berry, of Iowa City, Holly O'Berry, of Van Nuys, Calif., Danny O'Berry, of Beuren, Germany, Andrew O'Berry, Toby (Kelly) O'Berry, of Des Moines, and foster son, Bob Greene, along with numerous international and Iowa State students who lived with the family and became "adopted" children. They have seven grandchildren, Keegan, Keelee, Ely, Alice, Adacus, Harper and Hadley.

He was preceded in death by his parents, Luther and Marjorie O'Berry; brother, Joe; and son, Eric.

His career with Agriculture Research Service in the U.S. Dept. of Agriculture began in 1955 as a lab technician in Beltsville, Md. Forty-eight years later he retired having served as a research veterinarian, Director of the National Animal Disease Center and National Technology Transfer Coordinator.

Phil will be remembered for his volunteer service to the Ames and Des Moines communities. He was a 34 year member of the Board of Directors of Youth and Shelter Services where he filled all the offices from treasurer to president several times, was president of the Ames United Way Board of Directors, president of Mainstream Living Board of Directors, president of Mary Greeley Medical Center Board of Directors, president of Noon Kiwanis Club where he was a 40 year member, the Ames Chamber of Commerce Board of Directors, Youth and Shelter Services Foundation Board of Directors, and Phi Delta Theta Fraternity. Phil was a founding member of the Iowa Runaway, Homeless and Missing Youth Center Board of Directors in Des Moines.

Phil's membership in professional societies and honoraries is extensive. He was far too humble to want a list printed nor would he want the awards and honors to be mentioned. However, the plaque on his office wall honoring him as the City of Ames Citizen of the Year in 2000 as well as the expressions of appreciation for co-chairing or participating in four YSS (Youth and Shelter Services) capital campaigns raising over $8,850,000 gave him great satisfaction. Phil was an active member in the Conference of Research Workers in Animal Diseases.

Phil loved beach combing, bird watching, riding his recumbent bike at the Root River trail in Minnesota, pulling weeds in Terri's gardens, and wearing flip flops. He enjoyed a special fondness for cows. His family and the time he spent in volunteer service were the joys of his life. He was passionate about mentoring through Big Brothers of America, and Youth and Shelter Services. For over 45 years, he mentored many youth in Maryland and Iowa.

PROGRAM

CRWAD THANKS THE FOLLOWING 2011 SPONSORS

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Chicago Marriott, Downtown Magnificent Mile, Chicago, Illinois
December 4-6

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

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. 2011-65119-20597 .

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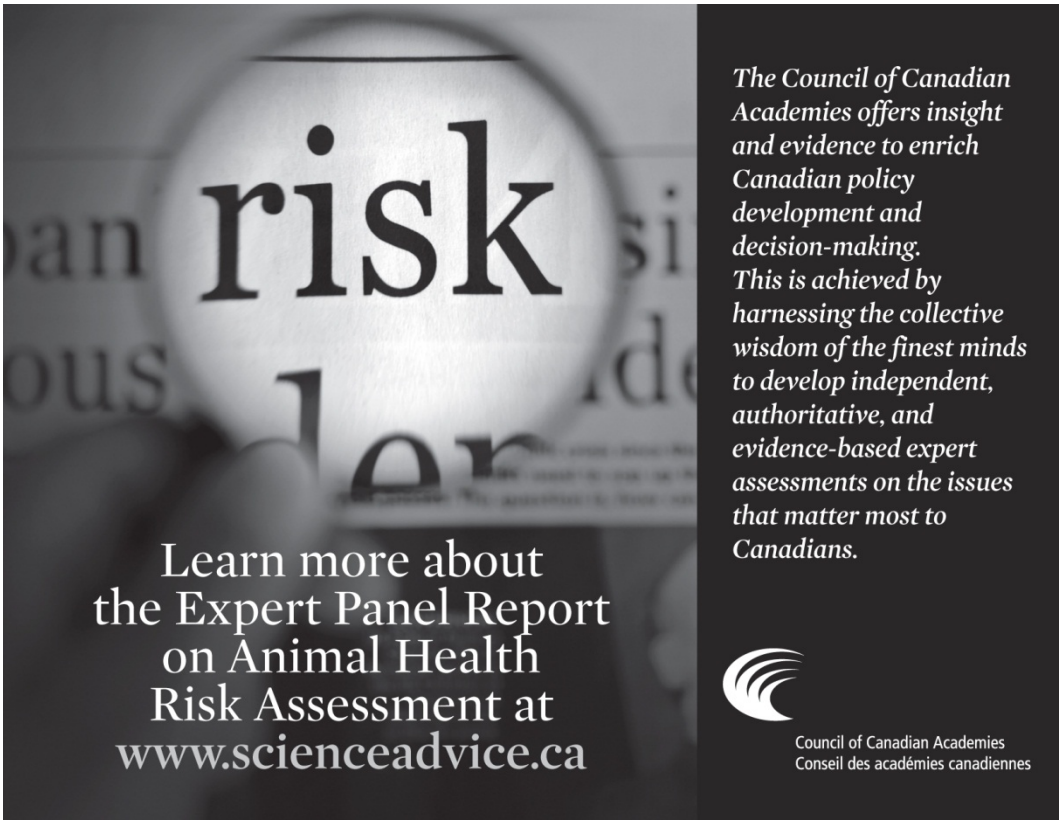

Exhibitors:

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2011 CRWAD Keynote Speakers and Titles

Bacterial Pathogenesis Section – Dr. Lisa K. Nolan
Professor & Dr. Stephen G. Juelsgaard Dean, Iowa State University, Ames, IA
Monday, December 5, 10:45 AM - Avenue Ballroom, 4th Floor
No. 007 - Title – Exploring APEC virulence, evolution and host specificity using pathogenomic approaches.

Biosafety and Biosecurity Section – Dr. Steven C. Olsen
Infectious Bacterial Diseases of Livestock Research Unit, National Animal Disease Center, Ames, IA
Monday, December 5, 3:00 PM - Denver/Houston Room, 5th Floor
No. 030 - Title - Working with Biolevel 3 Agents that interface across human, livestock, and wildlife boundaries.

Companion Animal Epidemiology, Epidemiology & Animal Health Economics, and Food & Environmental Safety Sections – Dr. Annette O'Connor
Iowa State University
Tuesday, December 6, 8:00 AM - Salons E, 5th Floor
No. 063 - Title - Epidemiologists: we're not the same as statisticians.

Gastroenteric Diseases Section – Dr. Philip Griebel
Vaccine and Infectious Disease Organization (VIDO), University of Saskatchewan, Saskatoon, SK, Canada
Monday, December 5, 8:45 AM – Michigan/Michigan State Room, 6th Floor
No. 104 - Title - Mucosal immune system development in the small intestine of the newborn calf: regional differences in innate and acquired immunity.

Immunology Section – Distinguished Veterinary Immunologist – Dr. Patricia E. Shewen
University of Guelph, Guelph, Ontario, Canada
Monday, December 5, 1:30 PM - Salons F/G/H, 5th Floor
No. 121 - Title - Immunology and Animal Health: the whole is greater than the sum of its parts.

Respiratory Diseases - Dr. Steeve Giguere
University of Georgia, Athens, GA
Monday, December 5, 3:45 PM - Indiana/Iowa Room, 6th Floor
No. 157 - Title - Infections caused by Rhodococcus equi in foals: Immunologic and therapeutic considerations.

Vector-Borne and Parasitic Diseases – Dr. John B. Dame
Infectious Diseases and Pathology, University of Florida, Gainesville, FL
Monday, December 5, 10:00 AM - Denver/Houston Room, 5th Floor
No. 164 - Title - Plasmepsins: Dissecting the function of a parasite's protease degradome.

Viral Pathogenesis Section – Dr. David L. Suarez
Research Leader EEAV, Southeast Poultry Research Laboratory, Athens, GA
Monday, December 5, 8:00 AM - Los Angeles/Miami/Scottsdale, 5th Floor
No. 169 - Title – DIVA Vaccination for Avian Influenza Virus: Ready for Prime Time?

2011 CRWAD - Keynote Speaker - Bacterial Pathogenesis Section

Lisa K. Nolan, DVM, PhD
Professor and Dr. Stephen G. Juelsgaard Dean, CVM, Iowa State University, Ames, IA

Abstract No. 007 - Title: Exploring APEC virulence, evolution and host specificity using pathogenomic approaches.

Dr. Lisa K. Nolan became the Dr. Stephen G. Juelsgaard Dean of Veterinary Medicine and the college's 15th dean in January, 2011. She has been a professor, administrator and researcher at Iowa State University since 2003, having served most recently as Associate Dean for Research and Graduate Studies since 2009. Dr. Nolan also served as Associate Dean for Academic and Student Affairs, Chair of the Department of Veterinary Microbiology and Preventive Medicine and Interim Chair of the Department of Veterinary Diagnostic and Production Animal Medicine.

She has gained international recognition as a researcher for her rigorous work in the area of *E. coli* that cause disease in animal and human hosts and holds three U.S. patents. In recent years, Dr. Nolan has given several invited presentations around the world, authored numerous refereed journal articles and book chapters, garnered millions of dollars in research funds, and served as an Associate Editor of the text *Diseases of Poultry* and on the advisory and editorial boards of the publication *Avian Diseases*.

She received her Doctor of Veterinary Medicine, Master of Science and Ph.D. degrees from the University of Georgia.

2011 CRWAD - Keynote Speaker - Biosafety and Biosecurity Section

Dr. Steven C. Olsen
Infectious Bacterial Diseases, USDA-ARS-NADC, Ames, IA

Abstract No. 030 - Title: Working with Biolevel 3 Agents that interface across human, livestock, and wildlife boundaries.

Dr. Steven C. Olsen is the current Research Leader and Veterinary Medical Officer in the Bacterial Diseases of Livestock Research Unit at the National Animal Disease Center (NADC) in Ames, Iowa. He has been at NADC since 1994. Prior experience included food animal, exotic, small animal and equine veterinary practices. His research group is conducting research to identify immunogenic peptides of *Brucella abortus*, define immunological mechanisms of *Brucella* infection, and develop new brucellosis vaccines for cattle and free-ranging wildlife, and complementary diagnostic tests. Dr. Olsen earned his BS, MS, DVM and PhD degrees at Kansas State University. He is a Diplomate of the American College of Veterinary Microbiologists (subspecialty Immunology), and has received numerous awards for his animal disease research. Dr. Olsen has published over 20 first author refereed manuscripts, three book chapters, and has been a frequent national and international invited speaker, especially on topics related to Brucellosis in domestic and wild ruminants.

2011 CRWAD - Keynote Speaker for the Companion Animal Epidemiology Section, Epidemiology & Animal Economics Section and Food & Environmental Safety Section

Dr. Annette O'Connor
Department of Veterinary Diagnostics & Production Animal Medicine
Iowa State University, Ames, IA

Abstract No. 063 - Title: Epidemiologists: we're not the same as statisticians.

Dr. O'Connor is a veterinary epidemiologist, and she has applied the principles of that discipline to a diverse set of fields, including livestock diseases, food-borne pathogens of animal proteins and veterinary public health. The main area of focus of Dr. O'Connor's research has been to understand how researchers can use study design and research synthesis methodologies to translate primary research findings into decision support tools for agencies such as the National Pork Board and the European Union Food Safety Authority. Topics evaluated include pre-harvest food safety interventions, post-harvest interventions, zoonotic pathogens and the impact of proximity to confined animal operations on community health. This work includes understanding how standards of evidence translate from human medicine to food safety.

In conjunction with research synthesis, Dr. O'Connor has used traditional epidemiological research approaches so that decision makers can better understand the efficacy of control options for Salmonellae pre-harvest in swine, bovine respiratory disease in feedlot cattle and infectious bovine keratoconjunctivitis in beef calves. The combined impact of this work has been to provide producers, veterinarians, government agencies and industry groups with independent advice about the efficacy of relevant interventions. Dr. O'Connor is an author of the REFLECT statement (2010), a reporting guideline for interventions involving livestock and food safety outcomes (similar to the CONSORT statement).

Dr. O'Connor received a Bachelor of Veterinary Science (BVSc) from the University of Sydney in 1993, a Masters of Veterinary Science (MVSc) from the University of Queensland in 1997 and a Doctoral of Veterinary Science (DVSc) from the University of Guelph in 2001. In 2009, Dr. O'Connor was admitted as a Fellow of Epidemiology to the Australian and New Zealand College of Veterinary Scientists. Dr. O'Connor is Professor of Epidemiology at Iowa State University. Dr. O'Connor teaches epidemiology methods and inference in the Preventive Veterinary Medicine Program at Iowa State University the MPH program at the College of Public Health at the University of Iowa.

2011 CRWAD - Keynote Speaker for the Gastroenteric Diseases Section

Philip J. Griebel, D.V.M., Ph.D.
Program Manager and Senior Immunologist, Vaccine and Infectious Disease Organization (VIDO), University of Saskatchewan, Saskatoon, SK Canada

Abstract No. 104 - Title: Mucosal immune system development in the small intestine of the newborn calf: regional differences in innate and acquired immunity.

Highlights
In collaboration with researchers at the University of Saskatchewan, UBC, Simon Fraser, and the Sanger Institute Dr. Griebel has been involved in the high throughput analysis of genes regulating innate mucosal immune responses. These collaborators have generated novel bioinformatics tools and technology platforms to analyze cell signaling events which play a key role in regulating inflammation and host responses to infection.

Dr. Griebel has also worked closely with numerous pharmaceutical companies to evaluate and develop novel immune therapies and vaccine delivery technologies. Vaccine research has focused specifically on the development of mucosal vaccines and their application for prevention of diseases in the neonate.

Background
1988, Ph.D. (Viral Immunology), University of Saskatchewan
1981, DVM , WCVM, University of Saskatchewan
1977, BsC. (Hons), University of Victoria
Published
Over 100-peer-reviewed articles
15 review articles/books/book chapters
Patents
3 granted (co-applicant)
3 pending (co-applicant)
Honours
Tier I Canada Research Chair in neonatal Mucosal Immunology

2011 CRWAD - Keynote Speaker - Respiratory Diseases Section

Dr. Steeve Giguere
Large Animal Medicine, CVM, University of Georgia, Athens, GA

Abstract No. 157 - Title: Infections caused by Rhodococcus equi in foals: Immunologic and therapeutic considerations.

Dr. Giguère received his DVM degree from the University of Montreal in 1992. He then completed an Internship in Equine Medicine and Surgery at the same university. After finishing a residency in Large Animal Internal Medicine at New Bolton Center, University of Pennsylvania, he completed a PhD in Veterinary Microbiology and Immunology at the University of Guelph in Ontario, Canada. Dr. Giguère was a faculty member at the University of Florida for 10 years where he also acted as Director of the Hofmann Equine Neonatal Intensive Care Unit. Dr. Giguère joined the University of Georgia as a Professor and Marguerite Thomas Hodgson Chair in Equine Studies in August 2009. His clinical interests include general internal medicine, with a particular interest in equine respiratory disorders, cardiology, and neonatology. His research interests include *Rhodococcus equi* infections of foals, neonatal equine immunology, as well as pharmacokinetics and pharmacodynamics of antimicrobial agents. Dr. Giguère is a Diplomate of the American College of Veterinary Internal Medicine.

2011 CRWAD - Keynote Speaker - Vector-Borne and Parasitic Diseases Section

Dr. John B. Dame, Infectious Diseases and Pathology, University of Florida, Gainesville, FL

Abstract No. 164 - Title: Plasmepsins: Dissecting the function of a parasite's protease degradome.

John B. Dame is currently Professor and Chair of the Department of Infectious Diseases and Pathology, College of Veterinary Medicine, University of Florida, Gainesville. He received his BS degree in Chemistry (Florida State University, 1971) and his PhD degree in Biochemistry (University of Washington, Seattle, 1977) followed by postdoctoral training in membrane biochemistry in the Department of Pharmacology, University of North Carolina, Chapel Hill.

Subsequently he applied his training to studies of parasitic diseases, working five years as a Staff Fellow/Senior Staff Fellow in the Malaria Section of the Laboratory of Parasitic Diseases of NIAID, where he helped establish a laboratory to study the molecular biology of the malaria parasite. Dr. Dame's most prominent early work was to clone and sequence the gene encoding the circumsporozoite antigen of *Plasmodium falciparum*, subunits of which have been utilized for the production of the first human malaria vaccine.

Prior to joining the faculty of the University of Florida, he worked two years as a Research Microbiologist at the Animal Parasitology Institute, Agricultural Research Service, USDA, applying similar molecular techniques to the characterization of protozoan and helminth parasites of animals. At the University of Florida he applied these molecular approaches to studies of numerous heminth and protozoan parasite species before returning to malaria research to lead one of the first large-scale, genome-wide sequencing projects for the malaria parasite.

Dr. Dame's research interests have since been focused primarily on the molecular biology of the malaria parasite. His recent studies have been directed at utilizing molecular genetic methods to identify and characterize potential antimalarial drug targets including the plasmepsin family of proteases. During his career he has authored more than 90 peer-reviewed publications, numerous scientific reviews, abstracts and other research related publications.

2011 CRWAD - Keynote Speaker for the Viral Pathogenesis Section
And Distinguished Veterinary Microbiologist

David L. Suarez
Research Leader EEAV, Southeast Poultry Research Laboratory, Athens, GA

Abstract No. 169 - Title – DIVA Vaccination for Avian Influenza Virus: Ready for Prime Time?

Dr. David Lee Suarez obtained a degree in Veterinary Medicine in 1988 from Auburn University. He obtained his Post-doctorate degree from Iowa State University in Veterinary Microbiology in 1995. Dr. Suarez is board certified in the American College of Veterinary Microbiology in both Virology and Immunology. From 1988 to 1991, he worked as an Associate veterinarian at Quintard Veterinary Hospital in Anniston. He remains a licensed veterinarian in the state of Iowa. Dr. Suarez was a Post-doctoral Research Associate at the Plum Island Animal Disease Center, New York in 1995. He joined the Southeast Poultry Research Laboratory, Agriculture Research Service, USDA in 1995 as a Veterinary Medical Officer in 1995. In 2005, he became Research Leader of the Exotic and Emerging Avian Viral Disease Research Unit with the same institution. He was the Acting Laboratory Director June 2010 to October 2011. His primary research interests are avian influenza virus (AIV) and Newcastle disease virus (NDV).
Since 1996, he has held the position of Adjunct Instructor in the Department of Infectious Diseases, University of Georgia. He is actively involved with two FAO projects on avian influenza in Egypt and Indonesia related to capacity building for the diagnostic laboratory and vaccine efficacy and selection. Dr. Suarez also has numerous other international collaborative projects.

2011 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. 4, 10 AM - 5:30 PM
Monday, Dec. 5, 7:00 AM - Noon, 2 - 5 PM
Tuesday, Dec. 6, 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. 4, 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. 5 - 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. 4, **Board Meeting**
8 AM - 12 PM - Los Angeles Room - 5th Floor
Monday, Dec. 5, **Business Meeting and Luncheon**
11:30 AM - 1PM - Buca di Beppo Restaurant
For more information contact Gina Pighetti

American College of Veterinary Microbiologists (ACVM)
Examination - Denver/Houston Room - 5th Floor
Friday, Dec. 2, 12 PM - 8 PM
Saturday, Dec. 3, 8 AM - 9 PM

Sunday, Dec. 4, Denver/Houston Room - 5th 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. 6, 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 - "Inviting logic to the table: using epidemiology to resolve food safety and zoonotic disease controversies"
A Symposium Honoring the Legacy of Dr. Dale D. Hancock
(Association for Veterinary Epidemiology and Preventive Medicine)
Sunday, Dec. 4, 11:30 PM - 5 PM, Chicago Ballroom Salons F/G/H - 5th Floor

Formal presentation to Dr. Hancock will be during CRWAD Business Meeting, Tuesday, Dec. 6, 11:45 AM - 12:30 AM, Chicago Ballroom A/B/C/D, 5th Floor.
For more information contact H. Morgan Scott and Jan Sargeant.

2011 CRWAD AND SATELLITE MEETINGS
(Alphabetically listed)

AVEPM Business Meeting – Buffet Luncheon - Members only
(Association for Veterinary Epidemiology and Preventive Medicine)
Monday, Dec. 5, 11:30 AM - Great America Room - 6th Floor
For more information contact Jan Sargeant or Morgan Scott

Batista BIVI Meeting
Thursday, December 1, 7:30 AM - 4:00 PM, Michigan State Room, 6th Floor
For more information contact Leigh Ann Cleaver

BIVI Banff Meeting
Thursday, December 1, 7:30 AM - 7:00 PM, Indiana Room, 6th Floor
For more information contact Leigh Ann Cleaver

BIVI Global Team Meeting
Thursday, December 1, 8:00 AM - 5:00 PM, Illinois Room, 6th Floor
For more information contact Leigh Ann Cleaver

BIVI PRRS ARCE Seminar
Friday, December 2, 2011, 6:00 AM - 12 PM, Salon E, 5th Floor
For more information contact Leigh Ann Cleaver

CRWAD Council Meeting
Saturday, Dec. 3, 5:30 PM - 9 PM - Great America Room - 6th Floor

CRWAD Business Meeting
Tuesday, Dec. 6, 11:45 AM - 12:30 PM - Chicago Ballroom A/B/C/D - 5th Floor
Dedication of the Meeting, Introduction of New Members, and Graduate Student Competition Awards;
New member applicants and all students entered in the competition are invited and encouraged to attend.

CRWAD Sponsorship Committee Meeting
Saturday, Dec. 3, 4:00 – 5:30 PM, Minnesota Room – 6th Floor

Distinguished Veterinary Immunologist Lecture by Dr. Patricia E. Shewen
University of Guelph, Guelph, Ontario, Canada
Monday, Dec. 5, 1:30 PM - Salons F/G/H, 5th Floor
Title - Immunology and Animal Health: the whole is greater than the sum of its parts.

Elsevier Meetings
Editorial Board of Meeting #1
Sunday, Dec. 4, 7:30 AM - 9 AM - Lincolnshire Room, 6th floor 2011

Editorial Board of Meeting no. 2
Monday, Dec. 5, 7:30 AM - 9 AM - Minnesota Room, 6th Floor

Exhibitors - (Table Top) Saturday - Tuesday, Dec. 4-7, 5th Floor Foyer
8 AM – 6 PM - 5th Floor Foyer (close Tuesday, Dec. 6, 10 AM)
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2011 CRWAD AND SATELLITE MEETINGS
(Alphabetically listed)

International PRRS Symposium (IPRRSS)

Friday, Dec, 2, Salons E/F/G/H, 5th Floor - Registration required for attendance.
1:00-1:30 PM – NC229 Business Meeting
1:30-2:45 PM - NC229 Scientific Meeting: What works & what questions still need to be answered to obtain PRRSV elimination and control?
2:00-2:30 PM Poster Boards available for poster assembly in Salons F/G/H
3:00-4:45 PM – Novel Vaccine and Vaccination Strategy Workshop – sponsored by USDA PRRS CAP-2
5:00 PM – Opening Session IPRRSS, Keynote by Dr. Christopher Walker
6:15PM - 8 PM - First Poster Session and IPRRSS Reception/Mixer (cash bar)

Saturday, December 3, Salons E/F/G/H, 5th Floor - Registration required for attendance.
8 AM - 5 PM, Oral Presentations
9:40 AM - Second Poster Session
12:00 PM – Lunch buffet in the poster area
2:40 PM - Break
For more information contact Joan Lunney, Lisa J. Becton (NPB), Bob Rowland or X.J. Meng.

Members of the Pilot Sampling Program for Antimicrobial Resistance

Tuesday, Dec. 6, 1:30 PM – 5:00 PM
Great American I/II Room, 6th Floor
For more information contact Mary Torrence at Mary.Torrence@ars.usda.gov

NC-1041 Enteric Diseases of Swine and Cattle Meeting

Saturday, Dec. 3, 8 AM - 5 PM – Kansas City Room - 5th Floor
Sunday, Dec. 4, 8 AM - 12 PM - Kansas City Room - 5th Floor
Attendance is by invitation only.
For more information contact Linda Mansfield. (mansfie4@cvm.msu.edu)

Rushmore Conference

Saturday, Dec. 3, 2011, 8 AM – 5PM – Salon D – 5th Floor
Meeting
Saturday, December 3, 2011, 5 PM – 7PM – 6th Floor
Mini oral presentations
Sunday, Dec. 4, 2011, 8 AM – 12 PM – Salon D – 5th Floor

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

Schwabe Symposium: "Inviting logic to the table: using epidemiology to resolve disease controversies" *– A Symposium Honoring the Professional Legacy of Dr. Dale D. Hancock –*

The Association for Veterinary Epidemiology and Preventive Medicine (AVEPM) is pleased to announce the program for the 2011 Schwabe Symposium honoring the lifetime professional achievements of Dr. Dale D. Hancock. This symposium will be held in Chicago on Sunday, December 4, 2011, at the Chicago Marriott – Downtown Magnificent Mile, just prior to the opening of the Conference of Research Workers in Animal Diseases. No registration or fees are required to attend. All are welcome. Please make plans to join us for this important event.

11:30 Light buffet lunch (soup and salad) <u>for symposium attendees</u>

12:30 Introductory remarks

12:35 Title: "*BSE and vCJD: Emerging science, public pressure and the vagaries of policy-making*."
Will Hueston, Professor, Center for Animal Health and Food Safety, University of Minnesota

13:15 Title: "*Data viewed through rose-tinted glasses: the example of bighorn sheep pneumonia*."
Tom Besser, Professor, Department of Microbiology and Pathology, Washington State University

13:55 Title: "*Evidence-based infection control in clinical practice: If you buy clothes for the emperor, will he wear them*?"
Paul Morley, Professor, Department of Clinical Sciences, Colorado State University

14:35 Break and Refreshments

15:05 Title: "*The front lines of epidemiology: balancing the need for action with need for logic and information*."
Susan Lance, Food and Drug Administration Liaison to the Centers for Disease Control and Prevention, Atlanta, GA

<u>Keynote address:</u>

15:45 Title: "*Veterinary epidemiology: the cavalry of bio-medicine*."
Dale D. Hancock, Professor, Field Disease Investigation Unit, Washington State University

16:40 Panel Discussion

17:00 Adjourn

18:00 – 20:00 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 2011 honoree is:

Dale D. Hancock, DVM, PhD

Dr. Dale Hancock is Professor of Epidemiology in the Field Disease Investigation Unit (FDIU) at the Washington State University College of Veterinary Medicine. Dr. Hancock earned his BS (1974) and DVM (1975) from Texas A&M University, and completed an ambulatory food animal residency in 1975-6 at The Ohio State University. After a stint in private practice at Scott Veterinary Hospital in Stephenville TX (where his fame for feline practice expertise persists), he returned to The Ohio State University to join the Ohio Agricultural Research and Development Center to complete his MS (1981) and PhD (1983). His MS graduate work involved evaluation of the role of fermented colostrum in providing local passive immunity to neonatal calves. His PhD graduate work considered the epidemiology of diarrheal morbidity and mortality in dairy heifer calves.

After a short stint at Mississippi State University, he accepted a position as epidemiologist in the Field Disease Investigation Unit at Washington State University in 1984, where he has since remained. Dr. Hancock has taught epidemiology to veterinary students and undergraduates, as well as public health, data management and analysis, ecology of infectious disease, production medicine, large animal medicine, and population medicine.

He led numerous farm visits for field disease investigations for the FDIU, and many of these investigations included veterinary student training. In recent years, Dale has dedicated almost every weekend to voluntarily leading farm visits intended to introduce non-farm background veterinary students to agricultural animal production systems.

During his tenure at WSU, Dr. Hancock developed and led a pioneering research program in the field of pre-harvest food safety, especially focusing on *Escherichia coli* O157:H7. This research program defined many of the key epidemiologic features of that agent in diverse animal populations. His research has been guided by a healthy skepticism of 'magic bullets' and a firm belief that the most useful interventions are based on logical modifications of management practices. His research, funded by >$12 million in grants as principal investigator, plus >$1.2 million as co-investigator, has thus far resulted in over 150 refereed manuscripts.

While Dr. Hancock remained at Washington State University for much of his career, his influence extended more broadly to an entire generation of food animal clinicians through his publication entitled *Population Medicine News*. This periodical existed between 1988 through 1996; for much of that period issued on a bi-weekly basis. Population Medicine News covered a wide variety of epidemiologic concepts and ideas, reflecting many of Dale's broad-ranging interests. For example, a true random sampling of the archives reveals issues entitled '*Making population statistics real (and useful)*", 9 Nov 1992; "*Population gestalt*", 5 Aug 1991; "*On the use of serologic tests to diagnose disease*", 15 May, 1996; "*Sampling bias in disease surveillance*", 2 Oct 1989; "*Drug residues*", 23 Jan 1989; "*Troubleshooting milking systems*", 20 Aug 1990; "*Sensitivity analysis with a spreadsheet*", 1 April 1991; and "*Heuristic tools for temporal analysis*", 7 Dec 1992. Regular readers of these newsletters can attest to the fact that the material was always presented in a direct and interesting way, with real life examples, and of course with Dale's characteristic sense of humor.

For more information about AVEPM, the Schwabe Symposium or CRWAD, please visit the following web pages:

- http://www.cvm.uiuc.edu/avepm/
- http://www.cvmbs.colostate.edu/mip/crwad/

2011 CRWAD PROGRAM - BY THE DAY

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

Speaker Ready Room: (Section meeting rooms are listed inside front cover)
Streeterville Room (2nd floor) is available on Sunday, Dec. 4 - Monday, Dec. 5

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

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

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

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

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

PROGRAM - BY THE DAY

Friday - Saturday - Sunday (afternoon) - Symposiums
Symposiums Schedule:
Friday - Dec. 2, 1:30 PM - 8 PM - International PRRS Symposium Program
Saturday - Dec. 3, 8 AM - 5:00 PM - Int. PRRS Symposium Program
Saturday, Dec. 3, 8 AM - 5:00 PM - 4th Int. Rushmore Conference on Enteric Diseases
Sunday, Dec. 4, 8 AM - 12:00 PM - 4th Int. Rushmore Conference on Enteric Diseases
Sunday - Dec. 4, 11:30 AM - 5 PM - AVEPM Symposium Program - Open Attendance

CRWAD Meeting Begins Sunday (evening):
Notice: Section meeting rooms are listed inside front cover
Sunday - Dec. 4 - 6:00-8:00 PM - Kick-Off CRWAD Reception and Poster Session I
Monday - Dec. 5, 8:00 AM – CRWAD Sections begin in separate rooms simultaneously.
Tuesday - Dec. 6, 8:00 AM - CRWAD Sections begin in separate rooms simultaneously.
Tuesday – Dec. 6, 5:00 PM – 6:30 PM – Poster Session II

Time	Oral No.	Section	Monday By-The-Day Title
8:00	040	EPIDEMIOLOGY AND ANIMAL HEALTH ECONOMICS	Investigation of pig mortalities in Maha Sarakham Province, Thailand, October 2010.
8:00	072	FOOD AND ENVIRONMENTAL SAFETY	Piloting the future: results from a pilot study for changes in the animal sampling program for the national antibiotic resistance monitoring system (narms).
8:00	101	GASTROENTERIC DISEASES	Identification of PPK-1 and PPK-2 dependent transcriptome responses in Campylobacter jejuni.
8:00	109	IMMUNOLOGY	A specific CpG site demethylation in the IFN-gamma gene promoter region of different aged equine.
8:00	137	RESPIRATORY DISEASES	Adaptationof a commercial PRRS serum antibody ELISA to oral fluid specimens.
8:00	158	VECTOR-BORNE AND PARASITIC DISEASES	Development of a sheep model for studying pathogen vector interactions of Anaplasma phagocytophilum and Ixodes scapularis.
8:00	169	VIRAL PATHOGENESIS	Viral Pathogenesis Keynote:
8:15	041	EPIDEMIOLOGY AND ANIMAL HEALTH ECONOMICS	Adjusting disease freedom confidence for imperfect diagnostic accuracy: A review of the evidence for non-traditional diagnostic specimens tested for PRRSV.
8:15	073	FOOD AND ENVIRONMENTAL SAFETY	Prevalence and antimicrobial resistance of potential food safety pathogens on united states beef cow-calf operations
8:15	102	GASTROENTERIC DISEASES	Loop mediated isothermal amplification method for detection of Lawsonia intracellularis.
8:15	110	IMMUNOLOGY	The DNA promoter of the interferon gamma gene (Ifng) is hypermethylated in neonatal foals.
8:15	138	RESPIRATORY DISEASES	Diagnostic performance of a commercial PRRS serum antibody ELISA adapted to oral fluid specimens.
8:15	159	VECTOR-BORNE AND PARASITIC DISEASES	Potentially protective dual oxidase enzymes (Duox1 and Duox2) in T.foetus infected bovine endometrial cells in vitro.
8:30	042	EPIDEMIOLOGY AND ANIMAL HEALTH ECONOMICS	Modeling of porcine reproductive and respiratory syndrome virus infection in a pig herd.
8:30	074	FOOD AND ENVIRONMENTAL SAFETY	The use of multi-level model residuals for food animal disease surveillance.
8:30	103	GASTROENTERIC DISEASES	Lawsonia intracellularis increases Salmonella enterica levels in the intestines of pigs.
8:30	111	IMMUNOLOGY	The effect of flunixin meglumine on the equine immune response to vaccination.
8:30	139	RESPIRATORY DISEASES	Diagnostic performance of a commercial PRRS serum antibody ELISA adapted to oral fluid specimens: longitudinal response in experimentally-inoculated populations.
8:30	160	VECTOR-BORNE AND PARASITIC DISEASES	Understanding the basis of strain-restricted immunity to Theileria parva.

Time	Oral No.	Section	Monday By-The-Day Title
8:45	001	BACTERIAL PATHOGENESIS	Detecting differential proteome expression between pathogenic and commensal Staphylococcus aureus strains using SILAC.
8:45	043	EPIDEMIOLOGY AND ANIMAL HEALTH ECONOMICS	Baseline study in the Niagara region porcine reproductive and respiratory syndrome (PRRS) area regional control and elimination project (ARC&E).
8:45	075	FOOD AND ENVIRONMENTAL SAFETY	Vaccination to control Escherichia coli O157 in integrated cattle production systems.
8:45	104	GASTROENTERIC DISEASES	Gastroenteric Diseases Section Keynote: Mucosal immune system development in the small intestine of the newborn calf: regional differences in innate and acquired immunity.
8:45	112	IMMUNOLOGY	The maturation of equine infectious anemia virus (EIAV) envelope-specific immune responses in vivo after exposure to a live-attenuated vaccine.
8:45	140	RESPIRATORY DISEASES	Evaluation of herd exposure methods to produce PRRSv-negative pigs from infected breeding herds.
8:45	161	VECTOR-BORNE AND PARASITIC DISEASES	Prevalence of tick-borne anaplasmal pathogens among naturally infected client-owned dogs in Missouri.
8:45	170	VIRAL PATHOGENESIS	Pathogenicity and cytokine gene expression patterns associated with fowl adenovirus serotype 4 infection.
8:45		BACTERIAL PATHOGENESIS	Session Presiders
9:00	002	BACTERIAL PATHOGENESIS	Staphylococcus aureus virulence cluster agr- regulated hemolysins dictate bovine polymorphonuclear leukocyte inflammatory signaling.
9:00	044	EPIDEMIOLOGY AND ANIMAL HEALTH ECONOMICS	Association between PRRSV ORF5 genetic distance and differences in space, time, ownership, and animal sources among commercial pig herds.
9:00	076	FOOD AND ENVIRONMENTAL SAFETY	Prevalence of Shiga toxin-producing Escherichia coli (STEC) genes by multiplex PCR in cattle and their environment, Michigan 2011.
9:00	113	IMMUNOLOGY	T regulatory cells and IgE are inversely correlated in horses vaccinated with viral vaccines.
9:00	141	RESPIRATORY DISEASES	Evaluation of immune responses to porcine reproductive and respiratory syndrome virus in pigs at day two post-infection.
9:00	162	VECTOR-BORNE AND PARASITIC DISEASES	Epidemiology of epizootic hemorrhagic disease in white-tailed deer in Texas.
9:00	171	VIRAL PATHOGENESIS	Genetic characterization of Newcastle disease viruses, allocated in Ukraine in 2006-2009.
		(continued)	

Time	Oral No.	Section	Monday By-The-Day Title
9:15	003	BACTERIAL PATHOGENESIS	Profile of vasoactive lipid mediators in S. uberis mastitis.
9:15	045	EPIDEMIOLOGY AND ANIMAL HEALTH ECONOMICS	The association between the PCVAD outbreak in Ontario and the positivity of Porcine Reproductive and Respiratory Syndrome virus ELISA and PCR test results.
9:15	077	FOOD AND ENVIRONMENTAL SAFETY	Multiple-locus variable-nucleotide tandem repeat analysis of Escherichia coli O157:H7 evaluating isolate distribution on a closed feedlot facility, Wooster, OH.
9:15	114	IMMUNOLOGY	Deacylated polyethyleneimine and IL-15 expression constructs modulate humoral and cellular immune responses to DNA vaccination in horses.
9:15	142	RESPIRATORY DISEASES	Attenuation of a virulent North American PRRS virus isolate on CD163-expressing cell lines, and demonstration of efficacy against a heterologous challenge.
9:15	163	VECTOR-BORNE AND PARASITIC DISEASES	Functional analysis of tick genes differentially expressed in response to Anaplasma phagocytophilum infection.
9:15	172	VIRAL PATHOGENESIS	Misfolded Y145stop catalyzes the conversion of full prion protein.
10:00	004	BACTERIAL PATHOGENESIS	Metagenomic evaluation of culture-negative clinical mastitis samples.
10:00	046	EPIDEMIOLOGY AND ANIMAL HEALTH ECONOMICS	The impact of maternally derived immunity on influenza virus transmission in neonatal pig populations.
10:00	078	FOOD AND ENVIRONMENTAL SAFETY	Intimin type characterization of non-O157 Shiga toxin-producing Escherichia coli isolates.
10:00	105	GASTROENTERIC DISEASES	Transcriptional profiling of a pathogenic and an attenuated homologous Lawsonia intracellularis isolate during in vitro infection.
10:00	115	IMMUNOLOGY	Efficacy of attenuated Salmonella enterica serovar Typhimurium SA186, deleted of the zinc transporter ZnuABC, to control pig salmonellosis.
10:00	143	RESPIRATORY DISEASES	Antiviral effect of various mutagens against PRRS Virus.
10:00	164	VECTOR-BORNE AND PARASITIC DISEASES	Vector-Borne and Parasitic Diseases Keynote: Plasmepsins: Dissecting the function of a parasite’s protease degradome.
10:00	173	VIRAL PATHOGENESIS	Pathogenicity and immune response in the lung of pigs experimentally infected with diverse genotype I PRRSV strains, including a pathogenic subtype 3 strain.
		(continued)	

Time	Oral No.	Section	Monday By-The-Day Title
10:15	005	BACTERIAL PATHOGENESIS	Chlamydia pecorum genital infection of dairy cows associates with significantly reduced fertility and decreased levels of circulating luteinizing hormone.
10:15	047	EPIDEMIOLOGY AND ANIMAL HEALTH ECONOMICS	Sensitivity of oral fluids for the detection of influenza virus in young pig populations with and without maternally derived immunity.
10:15	079	FOOD AND ENVIRONMENTAL SAFETY	Multistate Markov chain model to describe and compare fecal shedding dynamics of three Escherichia coli O157:H7 strains in cattle.
10:15	106	GASTROENTERIC DISEASES	A novel circular DNA virus from bovine stool is similar to chimpanzee stool-associated circular DNA virus, suggesting a new genus of circular DNA viruses.
10:15	116	IMMUNOLOGY	Modulation of MHC I & II and cytokine expression by EHV-1 ORF 1/2 at the respiratory epithelium
10:15	144	RESPIRATORY DISEASES	Lipid-anchored membrane association of the PRRS virus GP4 glycoprotein and co-localization with CD163 in lipid rafts.
10:15	174	VIRAL PATHOGENESIS	Sequence and virulence comparison of four North American isolates of porcine reproductive and respiratory syndrome virus.
10:30	006	BACTERIAL PATHOGENESIS	Genome scanning for conditionally essential genes in Salmonella.
10:30	048	EPIDEMIOLOGY AND ANIMAL HEALTH ECONOMICS	Detection of influenza A virus in aerosols from acutely infected pig populations.
10:30	080	FOOD AND ENVIRONMENTAL SAFETY	Characterization of Escherichia coli carrying bla_{CTX-M}isolated from fecal flora of dairy cattle.
10:30	107	GASTROENTERIC DISEASES	Quantitative evaluation of changes in C-reactive protein level and Salmonella enterica status as indicators of the swine health status in response to use of antibiotic growth promoter, Tylosin.
10:30	117	IMMUNOLOGY	Differential regulation of mucosal immune responses at various mucosal tissues in pigs infected with PRRSV strain VR2332.
10:30	145	RESPIRATORY DISEASES	Adaptation of a commercial blocking ELISA to the detection of antibodies against influenza A virus nucleoprotein (NP) in porcine oral fluid specimens.
10:30	175	VIRAL PATHOGENESIS	Isotype profile of PRRSV nucleocapsid-specific antibody response in pigs after experimental infection.
10:45	007	BACTERIAL PATHOGENESIS	Bacterial Pathogenesis Section Keynote: Exploring APEC virulence, evolution, and host specificity using pathogenomic approaches.
		(continued)	

Time	Oral No.	Section	Monday By-The-Day Title
10:45	049	EPIDEMIOLOGY AND ANIMAL HEALTH ECONOMICS	Influenza A virus genetic diversity in immune pigs.
10:45	081	FOOD AND ENVIRONMENTAL SAFETY	Validation of culture methods for non-O157 Shiga toxin-producing Escherichia coli.
10:45	108	GASTROENTERIC DISEASES	Does infection caused by a multidrug resistant organism influence antimicrobial use practices in equine colic patients that had surgery?
10:45	118	IMMUNOLOGY	Comparison of serological assays for Actinobacillus pleuropneumoniae (serotypes 1-9) on serum from pigs experimentally infected with APP or vaccinated with APP bacterins.
10:45	146	RESPIRATORY DISEASES	Emergence of novel reassortant H3N2 swine influenza viruses with the 2009 pandemic H1N1 genes in the United States.
10:45	165	VECTOR-BORNE AND PARASITIC DISEASES	Improvement in diagnostic specificity of anaplasma marginale msp5 epitope-based celisa with new antigen construct.
10:45	176	VIRAL PATHOGENESIS	Novel PRRSV ORF5a protein is not immunoprotective but drives GP5 glycosylation.
11:00	050	EPIDEMIOLOGY AND ANIMAL HEALTH ECONOMICS	Estimating hiding behavior for lameness in sows.
11:00	082	FOOD AND ENVIRONMENTAL SAFETY	Simulation model of vaccinating cattle against STEC O157 for pre-harvest food safety.
11:00	119	IMMUNOLOGY	Early vaccination of 5-day-old piglets does not alter the efficacy of two commercial porcine circovirus type 2 vaccines in an experimental triple infection challenge model.
11:00	147	RESPIRATORY DISEASES	Evaluation of cross-protection of FluSure XP® against a heterologous gamma cluster H1N1 swine influenza virus challenge.
11:00	166	VECTOR-BORNE AND PARASITIC DISEASES	A novel Theileria equi sporozoite challenge model for pathogenesis and immune control studies in immunocompetent and immunodeficient horses.
11:00	177	VIRAL PATHOGENESIS	Development of virus-like particle vaccine of porcine reproductive and respiratory syndrome virus and analysis of immune responses in the vaccinated mice.
11:15	051	EPIDEMIOLOGY AND ANIMAL HEALTH ECONOMICS	The effect of lameness and other morbidity causes on average daily gain in feedlot cattle.
11:15	083	FOOD AND ENVIRONMENTAL SAFETY	Virulence profiling of Shiga toxin-producing Escherichia coli O111:NM isolates from cattle.
11:15	120	IMMUNOLOGY	Nanoparticles entrapped killed PRRSV vaccine reduces PRRSV viremia in both homologous and heterologous PRRSV challenged pigs.
		(continued)	

Time	Oral No.	Section	Monday By-The-Day Title
11:15	148	RESPIRATORY DISEASES	Modified live virus vaccine induces a distinct immune response profile compared to inactivated influenza A virus vaccines in swine.
11:15	167	VECTOR-BORNE AND PARASITIC DISEASES	Light microscopic study of the developmental cycle of Ixodes scapularis.
11:15	178	VIRAL PATHOGENESIS	Intranasal delivery of an adjuvanted modified live porcine reproductive and respiratory syndrome virus vaccine reduces the ROS induced lung pathology.
11:30	168	VECTOR-BORNE AND PARASITIC DISEASES	Society for Tropical Veterinary Medicine Presentation
1:30	008	BACTERIAL PATHOGENESIS	Molecular characterization of Egyptian Escherichia coli strains isolated from broiler chickens and their environment.
1:30	025	BIOSAFETY AND BIOSECURITY	Biosecurity assessment as a tool towards risk-based surveillance on swine farms in southern Ontario.
1:30	052	EPIDEMIOLOGY AND ANIMAL HEALTH ECONOMICS	Risk factors for environmental contamination with Salmonella enterica in a veterinary teaching hospital.
1:30	084	FOOD AND ENVIRONMENTAL SAFETY	Meta-analysis of a three-dose regimen of a type III secreted protein vaccine for efficacy at reducing STEC O157 in feces of feedlot cattle.
1:30	121	IMMUNOLOGY	Immunology Section Keynote: Immunology and Animal Health: the whole is greater than the sum of its parts.
1:30	149	RESPIRATORY DISEASES	Evaluation of a commercial blocking ELISA kit for detection of influenza A nucleoprotein antibodies in canine sera.
1:30	179	VIRAL PATHOGENESIS	Effect of deoxynivalenol (DON) mycotoxin on porcine reproductive respiratory syndrome virus (PRRSV) in vitro.
1:45	009	BACTERIAL PATHOGENESIS	The siderophore esterases Fes and IroD contribute to virulence of avian extra-intestinal pathogenic Escherichia coli.
1:45	026	BIOSAFETY AND BIOSECURITY	Evaluation study of interventions for reducing the risk of PRRSV introduction into filtered farms via retrograde air movement (Back-drafting) through idle fans.
1:45	053	EPIDEMIOLOGY AND ANIMAL HEALTH ECONOMICS	Salmonella shedding in hospitalized horses with signs of colic, with or without diarrhea.
1:45	085	FOOD AND ENVIRONMENTAL SAFETY	Antimicrobial resistance in Escherichia coli recovered from feedlot cattle.
1:45	150	RESPIRATORY DISEASES	Pneumonia cases associated with Mycoplasma hyopneumoniae: a retrospective evaluation of diagnostic cases from 2003 to 2010.
		(continued)	

Time	Oral No.	Section	Monday By-The-Day Title
1:45	180	VIRAL PATHOGENESIS	FISHing for cats: development of a fluorescence in situ hybridization (FISH) assay targeting feline papillomaviruses.
2:00	010	BACTERIAL PATHOGENESIS	Genetic factors affecting the persistence and dissemination of bla_{CMY-2} positive IncA/C plasmids.
2:00	027	BIOSAFETY AND BIOSECURITY	Isolation of Salmonella organisms from the environment in a large animal hospital using electrostatic (Swiffer) and sterile sponge collection devices.
2:00	054	EPIDEMIOLOGY AND ANIMAL HEALTH ECONOMICS	Management practices associated with Salmonella or antimicrobial resistant Salmonella on United States dairy herds.
2:00	086	FOOD AND ENVIRONMENTAL SAFETY	Effects of a vaccine and a direct-fed microbial on fecal shedding of E. coli O157:H7 in pens of commercial feedlot cattle fed a diet supplemented with distiller’s grains.
2:00	151	RESPIRATORY DISEASES	Increased prevalence of torque teno viruses in porcine respiratory disease complex affected pigs.
2:00	181	VIRAL PATHOGENESIS	Sequence analysis of swine influenza viruses circulating in US before and after the pandemic 2009 H1N1 influenza outbreak.
2:15	011	BACTERIAL PATHOGENESIS	The Cia proteins of Campylobacter jejuni are delivered to the cytosol of host cells via a flagellar-dependent mechanism.
2:15	028	BIOSAFETY AND BIOSECURITY	A novel animal product-free avian cell line that supports high titer influenza virus replication.
2:15	055	EPIDEMIOLOGY AND ANIMAL HEALTH ECONOMICS	Phenotypic and genotypic characterization of methicillin-resistant Staphylococcus pseudintermedius in dogs, cats, and horses at a veterinary teaching hospital.
2:15	087	FOOD AND ENVIRONMENTAL SAFETY	Fecal shedding of Escherichia coli O26 in feedlot cattle from a field trial evaluating an Escherichia coli O157:H7 vaccine and a direct-fed microbial.
2:15	122	IMMUNOLOGY	The reduction of bacterial mastitis severity by treatment with 25-hydroxyvitamin D3.
2:15	152	RESPIRATORY DISEASES	The development and validation of two non-invasive diagnostic screening assays for the detection of tuberculosis infection in non-human primates.
2:15	182	VIRAL PATHOGENESIS	Identification of novel swine/pandemic H1N1 reassortant virus in pigs.
2:30	012	BACTERIAL PATHOGENESIS	Characterization of the methyl accepting chemotaxis proteins in Campylobacter jejuni.
		(continued)	

Time	Oral No.	Section	Monday By-The-Day Title
2:30	029	BIOSAFETY AND BIOSECURITY	Development of multiplex-PCR and electronic microarrays for detection and typing of avian and bovine high consequence viruses.
2:30	056	EPIDEMIOLOGY AND ANIMAL HEALTH ECONOMICS	Phenotypic and genotypic characterization of Methicillin-resistant Staphylococcus aureus in dogs, cats, and horses at a veterinary teaching hospital from 2007 to 2010.
2:30	088	FOOD AND ENVIRONMENTAL SAFETY	Development of a semi-quantitative ranking scheme to estimate the concentration of Escherichia coli O157:H7 in bovine feces.
2:30	123	IMMUNOLOGY	Mechanisms behind Mycobacterium avium subspecies paratuberculosis suppression of host cell apoptosis in primary bovine macrophages.
2:30	153	RESPIRATORY DISEASES	Comparison of peptide cocktails and purified protein derivatives for use in the Bovigam™ assay.
2:30	183	VIRAL PATHOGENESIS	In vitro reassortment between endemic H1N2 and pandemic 2009 H1N1 Swine Influenza Viruses
3:00	013	BACTERIAL PATHOGENESIS	In vitro characterization of equine source hyperimmune plasma against Bacillus anthracis toxins.
3:00	030	BIOSAFETY AND BIOSECURITY	Biosafety and Biosecurity Section Keynote: Working with Biolevel 3 Agents that interface across human, livestock, and wildlife boundaries.
3:00	057	EPIDEMIOLOGY AND ANIMAL HEALTH ECONOMICS	Using agent-based modeling of the village poultry sector in Thailand to identify opportunities for influenza transmission and potential interventions for disease control.
3:00	089	FOOD AND ENVIRONMENTAL SAFETY	Modeling the effect of bacterial transfer rates and interventions on the prevalence and concentration of Escherichia coli O157 on beef carcasses.
3:00	124	IMMUNOLOGY	Bovine macrophages produce extracellular traps in response to Mannheimia haemolytica and its leukotoxin.
3:00	154	RESPIRATORY DISEASES	Genome-wide analysis of gene expression profile change in response to BRSV and H. somni in bovine respiratory epithelial cells.
3:00	184	VIRAL PATHOGENESIS	Evaluation of cd25, foxp3, and ccl5 gene expression in placentomes of pregnant cattle inoculated with bovine viral diarrhea virus.
3:15	014	BACTERIAL PATHOGENESIS	Edwardsiella ictaluri cyclic adenosine 3’, 5’-monophosphate receptor protein (crp) mutant vaccine and boost immunization in catfish (Ictalurus punctatus).
		(continued)	

Time	Oral No.	Section	Monday By-The-Day Title
3:15	058	EPIDEMIOLOGY AND ANIMAL HEALTH ECONOMICS	Hierarchical Bayesian modeling of heteroskedasticity in average daily weight gain of feedlot cattle.
3:15	090	FOOD AND ENVIRONMENTAL SAFETY	Assessing risks of microbial contamination of produce from irrigation water.
3:15	125	IMMUNOLOGY	Impaired capacity of neutrophils to produce reactive oxygen species, release extracellular traps and express genes encoding for cytokines may contribute to altered immune function in periparturient dairy cows.
3:15	155	RESPIRATORY DISEASES	BRSV and H. somni synergy in bridging the alveolar barrier.
3:15	185	VIRAL PATHOGENESIS	PCV2 infection from birth through finishing.
3:30	015	BACTERIAL PATHOGENESIS	Identification of molecular targets for diagnosis of bovine tuberculosis.
3:30	059	EPIDEMIOLOGY AND ANIMAL HEALTH ECONOMICS	Culling decisions based on microbiological and serological test results for the control of Johne’s disease in beef cow-calf operations.
3:30	091	FOOD AND ENVIRONMENTAL SAFETY	Inactivation kinetic of feline calicivirus (norovirus surrogate) on lettuce by electron beam irradiation.
3:30	126	IMMUNOLOGY	Selenoproteins alter eicosanoid biosynthesis in macrophages.
3:30	156	RESPIRATORY DISEASES	Cytokine and chemokine responses of equine pulmonary alveolar macrophages are altered in a dose-dependent manner to Rhodococcus equi infection.
3:45	016	BACTERIAL PATHOGENESIS	Genomic signatures of M paratuberculosis from multiple animal species: a better understanding of Johne’s disease transmission.
3:45	060	EPIDEMIOLOGY AND ANIMAL HEALTH ECONOMICS	Associations of antimicrobial use and antimicrobial resistance in Escherichia coli isolates individually sampled from feedlot cattle.
3:45	092	FOOD AND ENVIRONMENTAL SAFETY	Effects of feeding copper sulfate, tetracycline and tylosin on the prevalence of transferable copper resistance gene, tcrB, among fecal enterococci of swine.
3:45	127	IMMUNOLOGY	Probing the effects of dual infections with Mycobacterium avium ss. paratuberculosis and bovine leukemia virus on Regulatory T cell prevalence and activity in cattle.
3:45	157	RESPIRATORY DISEASES	Respiratory Diseases Section Keynote: Infections caused by Rhodococcus equi in foals: immunologic and therapeutic considerations.
4:00	017	BACTERIAL PATHOGENESIS	Mycobacterium avium subspecies paratuberculosis produces spores.
		(continued)	

Time	Oral No.	Section	Monday By-The-Day Title
4:00	061	EPIDEMIOLOGY AND ANIMAL HEALTH ECONOMICS	Effect of intervention strategies on ceftiofur resistance determinant (blaCMY-2 gene) and its relationship with TetA and TetB genes in cattle.
4:00	093	FOOD AND ENVIRONMENTAL SAFETY	Export risk assessment for the export of deboned beef from South Africa to Egypt.
4:00	128	IMMUNOLOGY	Proinflammatory responses of bovine endothelial cells to non-esterified fatty acids.
4:15	018	BACTERIAL PATHOGENESIS	Molecular characterization of the Mycobacteria antigen 85 complex and Fibronectin interaction.
4:15	062	EPIDEMIOLOGY AND ANIMAL HEALTH ECONOMICS	Genotypic characterization and comparison of tetracycline-resistant Escherichia coli isolates arising from humans and swine in a vertically integrated agri-food system.
4:15	129	IMMUNOLOGY	Inhibition of Mycoplasma mycoides subsp. mycoides adherence to transformed bovine skin fibroblasts by Mycoplasma specific monoclonal antibodies.

Time	Oral No.	Section	Tuesday By-The-Day Title
8:00	019	BACTERIAL PATHOGENESIS	The structure of a calcium-binding leptospiral immunoglobulin-like protein (LigB) domain of Leptospira interrogans.
8:00	063	EPIDEMIOLOGY AND ANIMAL HEALTH ECONOMICS	Epidemiology and Animal Health Economics Section Keynote: Epidemiologists: we're not the same as statisticians.
8:15	020	BACTERIAL PATHOGENESIS	Evaluation of oral fluid samples as a diagnostic sample for detection of Erysipelothrix rhusiopathiae in pigs.
8:30	021	BACTERIAL PATHOGENESIS	Seroprevalence of brucellosis in sheep in the selected areas of Mymensingh and Netrakona districts of Bangladesh.
8:45	022	BACTERIAL PATHOGENESIS	Identification of antigens for more reliable Streptococcus equi specific ELISA assay.
8:45	031	COMPANION ANIMAL EPIDEMIOLOGY	Prevalence and risk factors for shedding of Campylobacter spp. in dogs that frequent dog parks in southern Ontario, Canada.
8:45	064	EPIDEMIOLOGY AND ANIMAL HEALTH ECONOMICS	Understanding geographic epidemiology: the geographic epidemiologic trillium.
8:45	094	FOOD AND ENVIRONMENTAL SAFETY	Detection of Salmonella enteritidis in poultry environmental samples using a pooled real-time PCR assay.
8:45	130	IMMUNOLOGY	Attenuation and protective efficacy of live attenuated Salmonella Gallinarum vaccines by employing a regulated delayed attenuation strategy.
9:00	023	BACTERIAL PATHOGENESIS	The role of Th17 cytokines, IL-17A and IL-22, during Listeria monocytogenes infection of the pregnant mouse.
9:00	032	COMPANION ANIMAL EPIDEMIOLOGY	Comparison of virus isolation, one-step real-time reverse transcriptase-PCR assay, and two rapid influenza diagnostic tests for detecting canine influenza virus (H3N8) shedding in dogs.
9:00	065	EPIDEMIOLOGY AND ANIMAL HEALTH ECONOMICS	Methodological comparisons for antimicrobial resistance surveillance in feedlot cattle.
9:00	095	FOOD AND ENVIRONMENTAL SAFETY	Genetic relatedness of Salmonella recovered from Michigan dairy farms in 2000 and 2009.
9:00	131	IMMUNOLOGY	Intranasal vaccination with Ad5-encoding influenza HA elicits sterilizing immunity to homologous challenge and partial protection to heterologous challenge in pigs.
9:15	024	BACTERIAL PATHOGENESIS	Diversity among clostridial isolates from cellulitis cases in turkeys.
9:15	033	COMPANION ANIMAL EPIDEMIOLOGY	Comparison of the geographical distribution of Feline Leukemia virus (FeLV) and Feline Immunodeficiency virus (FIV) infections in the United States (2000-2011).

Time	Oral No.	Section	Tuesday By-The-Day Title
9:15	066	EPIDEMIOLOGY AND ANIMAL HEALTH ECONOMICS	Multi-drug resistance in Ontario swine Streptococcus suis, Escherichia coli K88, and Pasteurella multocida isolates (1998 - 2010).
9:15	096	FOOD AND ENVIRONMENTAL SAFETY	Salmonella in lymph nodes of cattle presented for harvest.
9:15	132	IMMUNOLOGY	Cytotoxic T cell responses in the spleen of infectious bursal disease virus infected chickens.
10:00	034	COMPANION ANIMAL EPIDEMIOLOGY	Discussion: Help shape the future of companion animal epidemiology at CRWAD
10:00	067	EPIDEMIOLOGY AND ANIMAL HEALTH ECONOMICS	Effect of bovine Corona virus shedding and seropositivity on the risk for BRD in calves transitioning from the farm of origin to the feedlot.
10:00	097	FOOD AND ENVIRONMENTAL SAFETY	Stress-adaptation can influence virulence in Campylobacter jejuni.
10:00	133	IMMUNOLOGY	Antiviral regulation underlying the activation status of porcine monocytic innate immune cells.
10:15	035	COMPANION ANIMAL EPIDEMIOLOGY	Prevalence of and exposure factors for H3N8 canine influenza virus seropositivity in US dogs with influenza-like illness.
10:15	068	EPIDEMIOLOGY AND ANIMAL HEALTH ECONOMICS	Q-fever in small ruminants in Indiana.
10:15	098	FOOD AND ENVIRONMENTAL SAFETY	The relationship between the occurrence of Campylobacter in post-chill carcasses and flock prevalence at various sampling points in broiler production and processing.
10:15	134	IMMUNOLOGY	Differentiation and immunoregulatory characteristics of porcine lung mesenchymal stem cells.
10:30	036	COMPANION ANIMAL EPIDEMIOLOGY	Effect of Skin lessions on Haematological Picture of some Dogs in Ibadan.
10:30	069	EPIDEMIOLOGY AND ANIMAL HEALTH ECONOMICS	Seroprevalence of brucellosis and its association with other reproductive diseases in buffaloes in Bangladesh.
10:30	099	FOOD AND ENVIRONMENTAL SAFETY	Swine MRSA isolates form robust biofilms.
10:30	135	IMMUNOLOGY	Sandwich -ELISA for diagnostics of African Swine Fever.
10:45	037	COMPANION ANIMAL EPIDEMIOLOGY	Modeling feral cat population dynamics in Knox County, TN.
10:45	070	EPIDEMIOLOGY AND ANIMAL HEALTH ECONOMICS	Prevalence of Toxoplasma gondii in market age lambs in the United States.
10:45	100	FOOD AND ENVIRONMENTAL SAFETY	Longitudinal study of veterinary students for acquisition of methicillin-resistant Staphylococcus aureus associated with exposure to pork production facilities.
		(continued)	

Time	Oral No.	Section	Tuesday By-The-Day Title
10:45	136	IMMUNOLOGY	Fc expressed on the surface of the PED virus enhanced immunogenecity.
11:00	038	COMPANION ANIMAL EPIDEMIOLOGY	Using logistic regression models to create a scoring system for socialization status of shelter cats during a three day holding period.
11:00	071	EPIDEMIOLOGY AND ANIMAL HEALTH ECONOMICS	Prevalence of elevated temperatures among horses presented for importation to the United States.
11:15	039	COMPANION ANIMAL EPIDEMIOLOGY	Development and implementation of a Shelter Population Index (SPI) to evaluate population trends of cats and dogs in the United States.

POSTER PROGRAM

BACTERIAL PATHOGENESIS POSTERS

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

Section Leader: Gireesh Rajashekara

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

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

Name badge are required.

No.	Title	Authors
001P	Correlation of foot conformation with likelihood of development of digital dermatitis in lactating dairy cattle.	C. Haglund, A. Krull, **J. Rabenold**, P. Gorden, B. Leuschen, J. Shearer, P. Plummer; Iowa State University, Ames, IA, USA.
002P	The potential symbiotic relationship of anaerobic bacteria along with *Treponema* spp. in the development of papillomatous digital dermatitis.	**A. Krull**, J. Rabenold, M. Elliot, P. Gorden, J. Shearer, B. Leuschen, P. Plummer; Iowa State University, Ames, IA, USA.
003P	Identification and characterization of genes required for *Campylobacter* resistance to Fowlicidin-1, a chicken host defense peptide.	K.V. Hoang, **J. Lin**; University of Tennessee, Knoxville, TN, USA.
004P	Enzymatic characteristics of a novel enterobactin esterase Cee in Campylobacter.	**Y. Mo**, X. Zeng, J. Lin; Department of Animal Science, University of Tennessee, Knoxville, TN, USA.
005P	Characterization of enterobactin esterase Cee in *Campylobacter* led to a novel model of ferric enterobactin iron acquisition.	X. Zeng, F. Xu, Y. Mo, **J. Lin**; University of Tennessee, Knoxville, TN, USA.
006P	Complex molecular interaction of ferric enterobactin acquisition in *campylobacter*: role of TonB-ExbB-ExbB energy transduction system.	X. Zeng, F. Xu, **J. Lin**; University of Tennessee, Knoxville, TN, USA.
007P	Does *Campylobacter* use intracellular reductive iron release mechanism to utilize ferric enterobactin?	X. Zeng, **J. Lin**; University of Tennessee, Knoxville, TN, USA.
008P	Mutation of luxS gene in *Campylobacter jejuni* impacts major virulence attributes.	**K. Mou**, P. Plummer; Iowa State University, Ames, IA, USA.
009P	Identification of Iron Acquisition Machinery of Flavobacterium columnare.	**J. Santander**, L. Guan, M. Mellata, R.I. Curtiss; Arizona State University, Tempe, AZ, USA.
010P	Identification of *Streptococcus uberis* mutants susceptible to neutrophil killing.	**J. Warren**, O. Kerro Dego, R. Almeida, S.P. Oliver, G.M. Pighetti; University of Tennessee, Knoxville, TN, USA.
011P	Expression patterns of virulence genes of *E. coli* strains associated with acute and chronic bovineintramammary infections.	**O. Kerro Dego**, S.P. Oliver, R.A. Almeida; The University of Tennessee, Knoxville, TN, USA.
012P	Immunoproteome analysis of the outer membrane proteins of *Salmonella* Gallinarum.	**J. Sun**[1], J. Han[2], J. Jang[3], E. Hong[1], T. Hahn[1]; 1Kangwon national university, college of veterinary medicine, Chuncheon, Korea, Republic of, 2KBNP Technology Institute, KBNP Inc, yesan, Korea, Republic of, 3KBNP Technology Institute, KBNP Inc., yeasn, Korea, Republic of.
013P	*Salmonella* Enteritidis mutants with reduced Caco-2 cell invasiveness show impaired survival in chicken macrophages and reduced invasiveness in chicken liver cells.	H.-Y. Kim[1], **D.H. Shah**[2]; 1College of Veterinary Medicine, Western University of Health Sciences, Pomona, CA, USA, 2Department of Veterinary Microbiogy and Pathology, Washington State University, College of Veterinary Medicine, Pullman, WA, USA.
014P	Porcine circovirus ORF2 capsid protein express on the surface of a *Salmonella enterica typhimurium.*	**H. Jang**; Komipharm International Co. LTD., Gyeonggido, Korea, Republic of.

BACTERIAL PATHOGENESIS POSTERS

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

Section Leader: Gireesh Rajashekara

No.	Title	Authors
015P	Infection of C57BL/6 mice by *Trypanosoma musculi*, modulates host immune responses during *Brucella abortus* co-colonization.	**J.E. Lowry**[1], J. Leonhardt[2], C. Yao[2], E. Belden[2], G. Andrews[2]; 1Colorado State University, Fort Collins, CO, USA, 2University of Wyoming, Laramie, WY, USA.
016P	Comparison of abortion and infection after experimental challenge of pregnant bison and cattle with *Brucella abortus* strain 2308.	**S. Olsen**, C. Johnson; National Animal Disease Center, Ames, IA, USA.
017P	Evaluating the Biochemical Based Differentiation of *Bibersteinia trehalosi.*	S. Brace, C. Thompson, **G. Dewell**, P. Plummer, T. Frana; Iowa State University, Ames, IA, USA.
018P	Resolution of *Mycobacterium bovis* phylogeny using genome-wide single nucleotide polymorphisms.	**D.J. Joshi**[1], B. Harris[2], R. Waters[3], T. Thacker[3], M. Palmer[3], B. Mathema[4], B. Krieswirth[4], S. Sreevatsan[1]; 1University of Minnesota, St. Paul, MN, USA, 2USDA, APHIS, Ames, IA, USA, 3USDA, NADC, Ames, IA, USA, 4PHRI, NJ Medical School, Newark, NJ, USA.
019P	Lack of gut oxalate-degrading bacteria is a risk factor for calcium oxalate urolithiasis in cats.	**M.C. Figueiredo**, J. Gnanandarajah, J.E. Abrahante, J.P. Lulich, M.P. Murtaugh; University of Minnesota, Saint Paul, MN, USA.
020P	Bacterial community profiling of tonsils from diseased pigs using terminal restriction fragment length polymorphism analysis.	**S. Kernaghan**[1], S. Ojha[2], D. Slavic[2], S. Chen[2], Z. Poljak[3], J. MacInnes[1]; 1Department of Pathobiology, University of Guelph, Guelph, ON, Canada, 2Animal Laboratory Services, University of Guelph, Guelph, ON, Canada, 3Department of Population Medicine, University of Guelph, Guelph, ON, Canada.
021P	Regulation of Leptospira interrogans LenA protein.	**A. Verma**, B. Jutras, A. Chenail, B. Stevenson; University of Kentucky, Lexington, KY, USA.
022P	The embryo lethality of E*scherichia coli* isolates and its relationship to the presence of virulence-associated genes.	**Y.-K. Kwon**, M.S. Kang, J.Y. Oh, B.K. An, E.G. Shin, M.J. Kim, J.H. Kwon; Animal, Plant and Fisheries Quarantine and Inspection Agency, Anyang, Korea, Republic of.
023P	Putative adhesins of *Actinobacillus suis*.	A.R. Bujold[1], A. Kropinski[2], J. Nash[3], **J.I. MacInnes**[1]; 1University of Guelph, Guelph, ON, Canada, 2Laboratory for Foodborne Zoonoses, Guelph, ON, Canada, 3Public Health Agency of Canada, Toronto, ON, Canada.
024P	A case report describing detection of *Rhodoturola minuta* fungemia in an ewe lamb.	**C.G. Chitko-McKown**[1], K.A. Leymaster[1], M.P. Heaton, Jr.[1], D.D. Griffin[2], J.K. Veatch[3], S.A. Jones[1], M.L. Clawson[1]; 1USDA, ARS, U.S. Meat Animal Research Center, Clay Center, NE, USA, 2Great Plains Veterinary Educational Center, University of Nebraska-Lincoln, Clay Center, NE, USA, 3Breathitt Veterinary Center, Murray State University, Hopkinsville, KY, USA.

COMPANION ANIMAL EPIDEMIOLOGY POSTERS

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

Section Leaders: Margaret Slater and Laura Hungerford

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

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

Name badge is required.

No.	Title	Authors
025P	Seroprevalence of pandemic H1N1 and seasonal human influenza in domestic cats in Ohio.	**A. Ali**[1], J. Daniels[2], Y. Zhang[3], A. Rodriguez-Palacios[1], K. Hayes-Ozello[2], L. Mathes[2], C. Lee[1]; 1The Ohio State University, Wooster, OH, USA, 2The Ohio State University, Columbus, OH, USA, 3Animal Disease Diagnostic Laboratory, Ohio Department of Agriculture, Reynoldsburg, OH, USA.

EPIDEMIOLOGY AND ANIMAL HEALTH ECONOMICS POSTERS

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

Section Leader: Ashley Hill

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

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

Name badge is required.

No.	Title	Authors
026P	The use of nucleocapsid N and nsp7 proteins in PRRS genotype 1 virus diagnostics.	**V. Celer**, J. Jankova; University of Veterinary Sciences Brno, Brno, Czech Republic.
027P	Molecular epidemiology of highly pathogenic porcine respiratory and reproductive syndrome (hp-prrs) variants in southeast asian countries (Vietnam, Laos and Cambodia).	**H.M. Tun**[1], K. Inui[2], M. Shi[1], J. Wu[3], G.-H. Zhang[4], M. Liao[4], Z. Wei[5], F.C.C. Leung[1]; 1The University of Hong Kong, Hong Kong, Hong Kong, 2Food and Agricultural Organization of United Nations, Hanoi, Viet Nam, 3Division of Swine Diseases, Shandong Provincial Key Laboratory of Animal Disease Control & Breeding, Institute of Animal Science and Veterinary Medicine Shandong Academy of Agricultural Sciences, Jinan, China, 4Key laboratory of Animal Disease Control and Prevention, Ministry of Agriculture, College of Veterinary Medicine, South China Agricultural University, Guangzhou, China, 5Department of Swine Infectious Diseases, Shanghai Veterinary Research Institute, Chinese Academy of Agricultural Sciences, Shanghai, China.

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EPIDEMIOLOGY AND ANIMAL HEALTH ECONOMICS POSTERS

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

Section Leader: Ashley Hill

No.	Title	Authors
028P	Proposal for ongoing targeted surveillance in the PRRS area regional control and elimination (PRRS ARC&E) projects.	**Z. Poljak**[1], J. Carpenter[2], M. Misener[3], G. Charbonneau[3], B. Jones[3], J. Fairles[4], J. Alsop[5]; 1Department of Population Medicine, University of Guelph, Guelph, ON, Canada, 2OPIC OSHAB, Stratford, ON, Canada, 3South West Ontario Veterinary Services, Stratford, ON, Canada, 4Animal Health Laboratory, University of Guelph, Guelph, ON, Canada, 5Ontario Ministry of Agriculture, Food, and Rural Affairs, Guelph, ON, Canada
029P	Outbreak investigation of neonatal piglet's diarrhea disease in a county of China.	**X. Zheng, Sr.**; China Animal Health and Epidemiology Center, QingDao, China.
030P	Investigation of *Bacillus anthracis* Outbreak in the Central Part of Myanmar in 2010.	**Z.M.L. Latt, Jr.**; University of Veterinary Science, Yangon, Myanmar.
031P	Pathobiological Analysis of Bovine Viral Diarrhea Virus Identified in the Republic of Korea.	**K.-S. Choi**; Animal Science, Kyungpook National University, Sangju, Korea, Republic of.
032P	Brucellosis, an important Porcine Reproductive problem in Bangladesh.	**M.S. Rahman**, M.O. Rahman, N. Jahan; Bangladesh Agricultural University, Mymensingh, Bangladesh.
033P	A prevalence survey of bovine trichomoniasis in Mato Grosso do Sul, Brazil.	**A.J. Kling**[1], A.O. Pellegrin[2], R.S. Juliano[2], N.P. Zimmerman[2], I. Langohr[1], D. Agnew[1]; 1Michigan State University, East Lansing, MI, USA, 2Empresea Brasileira de Pesquisa Agropecuria-Pantanal, Corumba, Brazil.
034P	M. hyosynoviae and M. hyorhinis: a retrospective study.	**J.C. Gomes Neto**, P.C. Gauger, N. Boyes, E.I. Strait, D.M. Madson, K.J. Schwartz; Veterinary Diagnostic and Production Animal Medicine, Iowa State University, AMES, IA, USA.

FOOD AND ENVIRONMENTAL SAFETY POSTERS

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

Section Leader: Yvette Johnson-Walker

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
035P	Minimizing antibiotic resistance transmission: How are health goals and protocols communicated between workers and management on dairy farms?	**D.M. Short**[1], D.A. Moore[1], M.A. Davis[1], D.L. Moore[1], H.M. Gonzalez[2], J. Vanegas[3], K. Heaton[1], W.M. Sischo[1]; 1Washington State University, Pullman, WA, USA, 2Colorado State University, Fort Collins, CO, USA, 3Oregon State University, Corvallis, OR, USA

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FOOD AND ENVIRONMENTAL SAFETY POSTERS

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

Section Leader: Yvette Johnson-Walker

No.	Title	Authors
036P	Epidemiology of Shiga toxin-producing Escherichia coli (STEC) in swine.	**M. Tseng**[1], P. Fratamico[2], D. Manzinger[2], B. Garman[2], J. Funk[1]; 1Michigan State University, College of Veterinary Medicine, East Lansing, MI, USA, 2United States Department of Agriculture, Agricultural Research Service, Eastern Regional Research Center, Wyndmoor, PA, USA.
037P	Effects of corn-based distillers grain (dg) inclusion into feeding rations on the burden of *Escherichia coli* O157:H7 incommercial feedlot settings.	W.E. Chaney, G.H. Loneragan, **R.M. McCarthy**, M.F. Miller, B.J. Johnson, J.C. Brooks, M.M. Brashears; Texas Tech University, Lubbock, TX, USA.
038P	Prevalence of Shiga toxin-producing *Escherichia coli* serogroup O26 in feces of feedlot cattle.	**L. Schaefer**, Z. Paddock, C. Cull, X. Shi, S. Li, J. Bai, T. Nagaraja, D. Renter; Kansas State University, Manhattan, KS, USA.
039P	Prevalence, serotyping, and antimicrobial resistance profiling of *Salmonella* isolated from conventional and organic Oklahoma retail ground poultry.	A. Gad, K.K. Harclerode, **M.K. Fakhr**; The University of Tulsa, Tulsa, OK, USA.
040P	Loxoribine pretreatment reduces *Salmonella enteritidis* organ invasion in day-old chickens.	**C.L. Swaggerty**, H. He, K.J. Genovese, S.E. Duke, M.H. Kogut; USDA, College Station, TX, USA.
041P	Antimicrobial resistance in the ten most common S*almonella* serotypes at the S*almonella* bank at Washington State University, Pullman: 1986-2010.	**J.A. Afema**; Washington State University, Pullman, WA, USA.
042P	Detection of *Salmonella typhimurium* using dna barcode amplification assay.	**M. Kim**, A.S. Woubit, K. Srivastava, P. Reddy; Tuskegee University, Tuskegee, AL, USA.
043P	Prevalence of and antimicrobial resistance in E. coli and Salmonella isolated from raw chicken nuggets at retail purchased in Canada.	**N. Janecko**[1], B.P. Avery[1], R.J. Reid-Smith[1], P. Boerlin[2], S.A. McEwen[2]; 1Public Health Agency of Canada, Guelph, ON, Canada, 2University of Guelph, Guelph, ON, Canada.
044P	Virulence and antimicrobial resistance profiling of *Staphylococcus aureus* including MRSA strains isolated from retail meats sold in Oklahoma.	L. Abdalrahman, A. Stanley, **M.K. Fakhr**; The University of Tulsa, Tulsa, OK, USA.
045P	Detection of methicillin-resistant *Staphylococcus aureus* in swine oral fluids.	**A. Beahm**, J. Kinyon, L. Layman, L. Karriker, A. Ramirez, T. Frana; Dept. of Veterinary Diagnostic and Production Animal Medicine, Iowa State University, Ames, IA, USA.
046P	Effect of electron beam irradiation on *Mycobacterium avium* complex in ground pork.	**C.O. Thoen**, G.W. Beran, T.B. Bailey; Iowa State University, Ames, IA, USA.
047P	Comparative characteristics of listeria monocytogenes serotype 4b biological properties.	**V. Ushkalov, Esq.**, L. Vygovska; State Scientific Control Institute of Biotechnology and Strains of Microorganisms, Kyiv, Ukraine.

GASTROENTERIC DISEASES POSTERS

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

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

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

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

Name badge is required.

No.	Title	Authors
048P	Effect of dietary zinc supplementation (dietary ZnO and microencapsulated zinc oxide) on colibacillosis in weaned pigs.	**S. Kim**[1], J. Kim[2], C. Lee[1], N. Kim[1], D. Yang[1], J. Han[1]; 1Kangwon National University, Chuncheon, Korea, Republic of, 2CTC Bio Inc., Seoul, Korea, Republic of.
049P	The effect of bacteriophage on weaned pigs challenged with *Clostridium perfringens* type A and C.	**S. Kim**[1], J. Kim[2], N. Kim[1], C. Lee[1], D. Yang[1], J. Han[1]; 1Kangwon National University, Chuncheon, Korea, Republic of, 2CTC Bio Inc, Seoul, Korea, Republic of.
050P	The effect of bacteriophage on weaned pigs challenged with *Escherichia coli.*	**S. Kim**[1], J. Kim[2], D. Yang[1], C. Lee[1], N. Kim[1], J. Han[1]; 1Kangwon National University, Chuncheon, Korea, Republic of, 2CTC Bio Inc., Seoul, Korea, Republic of.
051P	The effect of bacteriophage on weaned pigs challenged with *Salmonella typhimurium.*	**S. Kim**[1], J. Kim[2], C. Lee[1], D. Yang[1], N. Kim[1], J. Han[1]; 1Kangwon National University, Chuncheon, Korea, Republic of, 2CTC Bio Inc., Seoul, Korea, Republic of.
052P	The effect of bacteriophage on weaned pigs challenged with *Escherichia coli.*	**S. Kim**[1], J. Kim[2], J. Han[1]; 1Kangwon National University, Chuncheon, Korea, Republic of, 2CJ Cheiljedang Corp, Seoul, Korea, Republic of.
053P	Detection of rotavirus genotypes and coronavirus among diarrheic and healthy beef and dairy calves from Buenos Aires Province, Argentina.	E.L. Louge Uriarte[1], V. Parreno[2], A. Badaracco[2], L. Garaicoechea[2], E. Spath[1], A.E. Verna[1], M.R. Leunda[1], F. Fernandez[2], **L. Saif**[3], A.C. Odeon[1]; 1Animal Health Group, EEA INTA, Balcarce, Argentina, 2Virology Institute, CICV y A INTA, Castelar, Argentina, 3Food Animal Health Research Program, OARDC, The Ohio State University, Wooster, OH, USA.
054P	Detection and molecular characterization of bovine noroviruses among bovine diarrhea cases in US Midwest.	**Y.-I. Cho**, J.-I. Han, D. Sun, S. Park, V. Cooper, K. Schwartz, K.-J. Yoon; Department of Veterinary Diagnostic and Production Animal Medicine, Iowa State University, Ames, IA, USA.
055P	Evaluation of a commercial rapid antigen detection kit for bovine enteric pathogens.	**Y.-I. Cho**, V. Cooper, G. Dewell, K. Schwartz, D. Sun, K.-J. Yoon; Department of Veterinary Diagnostic and Production Animal Medicine, Iowa State University, Ames, IA, USA.
056P	Secretion of heat-labile enterotoxin by porcine-origin enterotoxigenic *Escherichia coli* strains.	**P.R. Wijemanne**, R.A. Moxley; University of Nebraska-Lincoln, Lincoln, NE, USA.

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GASTROENTERIC DISEASES POSTERS

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

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

No.	Title	Authors
057P	A longitudinal study of *Campylobacter jejuni* infection in beef cattle and their environment.	**R.M. Mild**[1], M.A. Cooper[2], M.P. Ward[3], A.E. Armstrong[2], L.A. Griggs[2], K.K. Cooper[2], J.A. Marchello[4], G.C. Duff[4], B.F. Law[2], L.A. Joens[2]; 1Veterinary Science and Microbiology, University of Arizona, Tucson, AZ, USA, 2Veterinary Science and Microbiology, University of Arizona, Tucson, AZ, USA, 3Animal Sciences, University of Arizona, Tucson, AZ, USA, 4University of Sydney, Sydney, Australia.
058P	Short-term oral passive immunotherapy using anti-*Campylobacter jejuni* IgY does not reduce *C. jejuni* colonization from the intestinal tract of broiler chickens.	S. Al-Adwani[1], R. Crespo[1], M.E. Konkel[2], **D.H. Shah**[1]; 1Department of Veterinary Microbiology and Pathology, College of Veterinary Medicine, Washington State University, Pullman, WA, USA, 2School for Molecular Biosciences, Washington State University, Pullman, WA, USA.

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
059P	Swine toolkit progress for the us veterinary immune reagent network.	**J. Lunney**[1], P. Boyd[1], A. Crossman[1], J. LaBresh[2], L. Kakach[2], Y. Sullivan[2], B. Wagner[3], H. Dawson[4], D. Tompkins[5], E. Hudgens[5], C. Baldwin[5]; 1USDA ARS BARC APDL, Beltsville, MD, USA, 2Kingfisher Biotech, Inc., St. Paul, MN, USA, 3Cornell Univ., Ithaca, NY, USA, 4USDA ARS BARC DGIL, Beltsville, MD, USA, 5Univ. Massachusetts, Amherst, MA, USA.
060P	Innate immune responses of porcine intestinal epithelial cells (IPEC-J2) to virus-associated virulence determinants.	J. Joseph, A. Nelson, **R.S. Kaushik**; Biology and Microbiology Dept, South Dakota State University, Brookings, SD, USA.
061P	Serum neutralizing antibody titer is associated with PRRSV vaccination-induced protection against homologous and heterologous challenge.	N. Chen, X. Li, L. Pappan, B. Trible, M. Kerrigan, A. Beck, Y. Li, D. Hesse, F. Blecha, J.C. Nietfeld, R. Rowland, **J. Shi**; Kansas State University, Manhattan, KS, USA.
062P	Evaluation of a real-time PCR assay for pseudorabies virus surveillance purposes.	**L.C. Miller**[1], E.L. Zanella[2], K.M. Lager[1], T.T. Bigelow[3]; 1National Animal Disease Center-USDA-ARS, Ames, IA, USA, 2Universidade de Passo Fundo, Passo Fundo, Brazil, 3Veterinary Services-USDA, Des Moines, IA, USA.

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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
063P	The porcine antibody repertoire and its response to PRRSV infection.	**J.C. Schwartz**, J.E. Abrahante, M.P. Murtaugh; University of Minnesota, St. Paul, MN, USA.
064P	Toll-like receptors and pro-inflammatory cytokine expression profile of porcine intestinal epithelial cells upon stimulation with enterotoxigenic *Escherichia coli.*	C. Sreenivasan[1], M. Zhao[2], D. Francis[2], **R.S. Kaushik**[3]; 1Department of Biology and Microbiology, South Dakota State University, Brookings, SD, USA, 2Department of Veterinary and Biomedical Sciences, South Dakota State University, Brookings, SD, USA, 3Department of Biology and Microbiology, and Department of Veterinary and Biomedical Sciences, South Dakota State University, Brookings, SD, USA.
065P	Modulation of antimicrobial Host defense peptide gene expression by free fatty acids.	**L.T. Sunkara**[1], W. Jiang[1], M. Achanta[1], G. Zhang[2]; 1Animal Science, Oklahoma State University, Stillwater, OK, USA, 2Animal Science, Biochemistry & Molecular Biology, and Physiological Sciences, Oklahoma State University, Stillwater, OK, USA.
066P	Mucosal immunity in chickens infected with low-pathogenic avian influenza viruses.	**P. Shutchenko**, B. Stegniy, G. Krasnikov, M. Stegniy; National Scientific Center – Institute of Experimental and Clinical Veterinary Medicine, Kharkiv, Ukraine.
067P	Genome-wide histone methylation analysis in MD-resistant and MD-susceptible chickens after MDV infection.	**J. Luo**[1], A. Mitra[1], F. Tian[1], S. Chang[2], H. Zhang[2], K. Cui[3], Y. Yu[4], K. Zhao[3], J. Song[1]; 1University of Maryland, College Park, MD, USA, 2USDA, ARS, Avian Disease and Oncology Laboratory, East Lansing, MI, USA, 3National Institutes of Health, Bethesda, MD, USA, 4China Agricultural University, Beijing, China.
068P	Decreased permeability changes in bovine epithelial cells treated with P2X7 receptor antagonists prior to exposure to adenosine triphosphate.	**D.J. McClenahan**, M.R. Orr, R.D. Patel; University of Northern Iowa, Cedar Falls, IA, USA.
069P	Deciphering the impact of maternal cells in neonatal health and immune development.	**S.M. Neal**, W.A. Wark, S.N. Garst, C.S. Petersson-Wolfe, I. Kanevsky-Mullarky; Virginia Polytechnic Institute and State University, Blacksburg, VA, USA.
070P	Proteomic evaluation of protein modulation in bovine lung tissue following *in vivo* challenge with Mannheimia haemolytica pneumonia.	**E.A. Tall**, J.L. Ward, J.L. Boehmer; Center for Veterinary Medicine, Laurel, MD, USA.
071P	Expression of CXCR1 and CXCR2 in bovine mammary tissue.	**L. Siebert**[1], J. Lippolis[2], G. Pighetti[1]; 1University of Tennessee, Knoxville, TN, USA, 2USDA-NADC, Ames, IA, USA.

(continued)

IMMUNOLOGY POSTERS

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

Section Leader: Isis Mullarky

No.	Title	Authors
072P	Strain virulence affects *Mycobacterium bovis* - infected bovine macrophage gene expression.	Y. Villarreal-Morales[1], R. Mancilla-Jimenez[2], R. Hernandez-Pando[3], **J.A. Gutierrez-Pabello**[1]; 1Facultad de Medicina Veterinaria y Zootecnia, Universidad Nacional Autónoma de México, Mexico, D.F., Mexico, 2Instituto de Investigaciones Biomedicas, Universidad Nacional Autonoma de Mexico., Mexico, D.F., Mexico, 3Instituto Nacional de Ciencias Medicas y Nutricion Salvador Zubiran, Mexico, D.F., Mexico.
073P	The role of regulatory T cells in immune responses to *Mycobacterium avium* subspecies *paratuberculosis* (Johne's disease) in cattle.	**B.N. Murphy**[1], S.S. Sipkovsky[1], J.A. Roussey[2], C.J. Colvin[1], P.M. Coussens[1]; 1Department of Animal Science, College of Agriculture and Natural Resources, Michigan State University, East Lansing, MI, USA, 2Comparative Medicine and Integrative Biology Program, College of Veterinary Medicine, Michigan State University, East Lansing, MI, USA.
074P	*Salmonella* serovars differentially stimulate bovine leukocyte responses *in vitro.*	D. Pan[1], M.H. Rostagno[2], P.D. Ebner[1], **S.D. Eicher**[2]; 1Purdue University, West Lafayette, IN, USA, 2USDA-ARS, West Lafayette, IN, USA.
075P	Identification of immunorelevant (IR) genes in Brucella abortus infected bovine dendritic cells by suppression subtractive hybridization.	**M.C. Heller**[1], J.L. Watson[1], M.T. Blanchard[1], K.A. Jackson[1], J.L. Stott[1], R.M. Tsolis[2]; 1University of California Davis, School of Veterinary Medicine, Davis, CA, USA, 2University of California Davis, School of Medicine, Davis, CA, USA.
076P	Identification and characterization of molecules suitable for improved diagnostics for Contagious Bovine Pleuropneumonia (CBPP).	**N.W.G. Ngatiri**; International Livestock Research Institute, Nairobi, Kenya.
077P	How long are viruses detected by PCR in blood and nasal swabs of calves vaccinated with infectious vaccines?	**B. Thiel**[1], D. Sudbrink[1], C. Haase[1], L.J. Larson[1], S. Schultz[1], K. Kurth[2], R. Schultz[1]; 1University of Wisconsin-Madison, Madison, WI, USA, 2Wisconsin Veterinary Diagnostic Laboratory, Madison, WI, USA.
078P	Production of mycobacterial recombinant proteins ESAT-6 and CFP-10 for bovine tuberculosis diagnosis.	**S. Flores-Villalva**[1], F. Suarez-Guemes[1], C. Espitia[2], M. Vordermeier[3], J. Gutierrez-Pabello[1]; 1Facultad de Medicina Veterinaria y Zootecnia, UNAM, D.F., Mexico, 2Instituto de Investigaciones Biomedicas, UNAM, D.F., Mexico, 3TB-Research Group, Veterinary Laboratories Agency, Weybridge, Addlestone, Surrey., UK.
079P	The effect of vitamin E supplementation on an experimental Haemonchus Contortus infection in lambs.	K. Barron[1], B. De Wolf[1], A. Zajac[2], B. Sartini[1], **K. Petersson**[1]; 1University of Rhode Island, Kingston, RI, USA, 2Virginia-Maryland Regional College of Veterinary Medicine, Virginia Tech, Blacksburg, VA, USA.

(continued)

IMMUNOLOGY POSTERS

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

Section Leader: Isis Mullarky

No.	Title	Authors
080P	The effect of age on telomerase activity and reactive oxygen species (ROS) production in plasma and peripheral blood mononuclear cells of horses.	**A.A. Adams**, A. Simpson, D.W. Horohov; The Gluck Equine Research Center, Lexington, KY, USA.
081P	Antibody and antigen-specific intradermal response of foals and adult horses vaccinated with a live attenuated vaccine.	**T.L. Sturgill**, S. Gigure, L.J. Berghaus, M.K. Hondalus, D.J. Hurley; University of Georgia, Athens, GA, USA.
082P	Comparison of the ability of two different adjuvants to stimulate antigen presenting cells function *in vivo* .	**J. Dunham**, C. Liu, D.W. Horohov; University of Kentucky, Lexington, KY, USA.
083P	Neutralizing antibody-mediated control of equine infectious anemia virus infection in the absence of T lymphocytes.	**S. Taylor**[1], S. Leib[2], S. Carpenter[3], R. Nelson[2], R.H. Mealey[2]; 1Purdue University, Lafayette, IN, USA, 2Washington State University, Pullman, WA, USA, 3Iowa State University, Ames, IA, USA.
084P	How long do memory cells persist in the dog vaccinated with Leptospira canicola, icterohaemorraghiae, grippotyphosa, and pomona?	**L.J. Larson**[1], B. Thiel[1], O. Okwumabua[2], A. Olsen[3], R. VanDomelen[3], R. Schultz[1]; 1University of Wisconsin-Madison, Madison, WI, USA, 2Wisconsin Veterinary Diagnostic Laboratory, Madison, WI, USA, 3Ridglan Farms, Mount Horeb, WI, USA.
085P	Are recently vaccinated puppies protected from challenge with multiple isolates of canine distemper virus (CDV)?	**L.J. Larson**, B. Thiel, D. Sudbrink, P. Sharp, R. Schultz; University of Wisconsin-Madison, Madison, WI, USA.
086P	Infectious canine vaccines can cause PCR diagnostic tests to be positive for up to three weeks post vaccination.	**L.J. Larson**[1], B. Thiel[1], K. Kurth[2], R. Schultz[1]; 1University of Wisconsin-Madison, Madison, WI, USA, 2Wisconsin Veterinary Diagnostic Laboratory, Madison, WI, USA.
087P	Do current canine combination vaccines provide protection against challenge with multiple isolates of Canine Distemper Virus (CDV)?	**L.J. Larson**, B.E. Thiel, R.D. Schultz; University of Wisconsin-Madison, Madison, WI, USA.
088P	Animal anthrax spore vaccine with nanogold.	**V. Ushkalov, Esq.**, A. Machuskyy, L. Vygovska; State Scientific Control Institute of Biotechnology and Strains of Microorganisms, Kyiv, Ukraine.

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
089P	A survey of porcine reproductive and respiratory syndrome in wild boar populations in Korea.	**E.J. Choi**, C.H. Lee, B.H. Hyun, J.J. Kim, S.I. Lim, J.Y. Song, Y.K. Shin; Viral Disease Division, Animal, Plant and Fisheries Quarantine and Inspection Agency, Anyang, Korea, Republic of.
090P	Detection of porcine reproductive and respiratory syndrome virus using a Mie scattering immunoagglutination assay in a microfluidic chip.	**J.Y. Lee**, C.H. Lee, E.J. Choi, J.Y. Song; Viral Disease Division, Animal, Plant and Fisheries Quarantine and Inspection Agency, Anyang, Korea, Republic of.
091P	Effect of temperature and relative humidity on UV_{254} inactivation of airborne PRRSV.	T.D. Cutler[1], C. Wang[2], S.J. Hoff[3], **J.J. Zimmerman**[1]; 1Department of Veterinary Diagnostic and Production Animal Medicine, Iowa State University, Ames, IA, USA, 2Department of Veterinary Diagnostic and Production Animal Medicine, and Department of Statistics, Iowa State University, Ames, IA, USA, 3Department of Agricultural and Biosystems Engineering, Iowa State University, Ames, IA, USA.
092P	The regulatory role of the zinc finger motif of the PRRSV Nsp1α protein for IFN modulation.	**M. Han**, Y. Du, C. Song, D. Yoo; University of Illinois at Urbana-Champaign, Urbana, IL, USA.
093P	Establishment of a stable cell line expressing non-structural protein 11 of PRRSV.	**D. Li**, Y. Sun, C. Song, D. Yoo; University of Illinois at Urbana-Champaign, Urbana, IL, USA.
094P	Different immunobiological features of two genetically distinct type 2 PRRS Viruses.	**A. Khatun**[1], E.-J. Choi[2], C.-H. Lee[2], D. Sun[3], K.-J. Yoon[3], W. Kim[1]; 1Chonbuk National University, Jeonju, Korea, Republic of, 2Animal Plant Fisheries Quarantine and Inspection Agency, Anyang, Korea, Republic of, 3Iowa State University, Ames, IA, USA.
095P	Development of DNA vaccine against H1N1 subtype swine influenza viruses.	**H. Wei**, S.D. Lenz, D.H. Thompson, R.M. Pogranichniy; Purdue University, West Lafayette, IN, USA.
096P	Porcine respiratory disease complex assessment using a slaughterhouse lung-scoring method in Korea.	**N. Kim**, S. Kim, C. Lee, D. Yang, J. Han; Kangwon national university, chuncheon, Korea, Republic of
097P	An investigation of lung lesions and pathogens associated with porcine respiratory disease complex in Korea.	**C. Lee**, S. Kim, N. Kim, D. Yang, J. Han; Kangwon national university, chuncheon, Korea, Republic of.
098P	Serotype and antimicrobial susceptibility of *Actinobacillus pleuropneumoniae* isolated from pigs in Korea.	**J. Jung**; Komipharm, Gyeonggido, Korea, Republic of.

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
099P	Emergence of a new swine H3N2 and pandemic H1N1 2009 influenza A virus reassortant in two Canadian animal populations: mink and swine.	**C. Bellehumeur**[1], D. Tremblay[1], V. Allard[1], J.-F. Doyon[2], G.J. Spearman[3], J. Harel[1], C.A. Gagnon[1]; 1Universite de Montreal, St-Hyacinthe, QC, Canada, 2Veterinary Clinic Jean-Francois Doyon, Roxton Falls, QC, Canada, 3Nova Scotia Department of Agriculture, Truro, NS, Canada.
100P	Gene microarray analysis of bovine bronchial epithelial cells exposed to bovine herpesvirus-1 or *Mannheimia haemolytica* in vitro.	A. N'jai, J. Rivera-Rivas, **K. Owusu-Ofori**, C. Czuprynski; UW-Madison, Madison, WI, USA.
101P	Variability of *Mannheimia spp.* in ruminant respiratory disease.	**K.A. Clothier**; University of California, Davis, Davis, CA, USA.
102P	Isolation of *Rothia nasimurium* from the lungs of CFTR-knockout ferrets modeling cystic fibrosis.	**T. Frana**[1], J. Kinyon[1], X. Sun[2], H. Sui[2], B. Liang[2], Y. Yi[2], W. Zhou[2], Y. Zhang[2], J.F. Engelhardt[2]; 1Dept. of Veterinary Diagnostic and Production Animal Medicine, Iowa State University, Ames, IA, USA, 2Dept. of Anatomy and Cell Biology, University of Iowa, Iowa City, IA, USA.

VECTOR-BORNE AND PARASITIC DISEASES POSTERS

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

Section Leader: Roman Ganta

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

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

Name badge is required.

No.	Title	Authors
103P	Outbreak of bovine epizootic encephalomyelitis by Akabane virus infection in Korea.	**J.-K. Oem;** Animal, Plant and Fisheries Quarantine and Inspection Agency, Anyang, Korea, Republic of.
104P	Laboratory-based reporting of cattle diseases in the upper northeast part of Thailand in 2009.	**P. Srisai**[1], S. Polpak[2], M. Polpak[2], P. Punnurit[2], K. Chanachai[1]; 1International Field Epidemiology Training Program for Veterinary, Bangkok, Thailand, 2Upper Northeast Veterinary Research and Development Center, Khon Kaen, Thailand.
105P	A universal quantitative PCR assay for tick-borne Anaplasmatacae infections.	**K.M. Steele**; University of Missouri, Columbia, MO, USA.

VIRAL PATHOGENESIS POSTERS

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

Section Leader: Kyoung-Jin Yoon

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

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

Name badge is required.

No.	Title	AuthorBlock
106P	Development of highly pathogenic avian influenza and infectious bronchitis viruses plasmid control positive templates for polymerase chain reaction.	**A.P. Gerilovych**, B.T. Stegniy, O. Solodyankin; NSC Institute of experimental and clinical veterinary medicine, Kharkiv, Ukraine.
107P	Antigenic properties of multicomponent inactivated vaccines against highly pathogenic avian influenza and Newcastle disease.	**B. Stegniy**, D. Muzyka, A. Stegniy; National Scientific Center – Institute of Experimental and Clinical Veterinary Medicine, Kharkiv, Ukraine.
108P	Expression of recombinant glycoprotein D of *Canine herpesvirus* 1 in bacterial cells.	**M. Vankova**, V. Celer; University of Veterinary Sciences Brno, Brno, Czech Republic.
109P	Monitoring of Newcastle disease and the other Paramyxoviruses among wild birds and poultry in Ukraine in 2000-2010.	B. Stegniy, **P. Shutchenko**; National Scientific Center – Institute of Experimental and Clinical Veterinary Medicine, Kharkiv, Ukraine.

ORAL PROGRAM

BACTERIAL PATHOGENESIS

Avenue Ballroom - 4th Floor

Section Leader: Gireesh Rajashekara

		Presiders: Gireesh Rajashekara and Jun Lin	
Time	**No.**	**Title**	**Authors**
8:45 Mon.	**001**	Detecting differential proteome expression between pathogenic and commensal Staphylococcus aureus strains using SILAC.	**M. Manickam**, I.K. Mullarky; Virginia Polytechnic Institute and State University, Blacksburg, VA, USA.
9:00	**002**	*Staphylococcus aureus* virulence cluster *agr*- regulated hemolysins dictate bovine polymorphonuclear leukocyte inflammatory signaling.	**R.J. Ortiz Marty**, W. Wark, I. Mullarky; Virginia Tech, Blacksburg, VA, USA.
9:15	**003**	Profile of vasoactive lipid mediators in *S. uberis* mastitis.	**C.M. Corl**[1], J. Lippolis[2], L. Sordillo[1]; 1Michigan State University, East Lansing, MI, USA, 2National Animal Disease Center, ARS, USDA, Ames, IA, USA.
9:30		**Break and Table Top Exhibits – Foyer**	
		Presiders: Gireesh Rajashekara and Qijing Zhang	
10:00	**004**	Metagenomic evaluation of culture-negative clinical mastitis samples.	J. Kuehn[1], P. Gorden[1], D. Monro[2], C. Wang[1], Q. Dong[2], G. Phillips[1], **P. Plummer**[1]; 1Iowa State University, Ames, IA, USA, 2University of North Texas, Denton, TX, USA.
10:15	**005**	*Chlamydia pecorum* genital infection of dairy cows associates with significantly reduced fertility and decreased levels of circulating luteinizing hormone.	**A. Poudel**[1], S. Rahman[1], E. Newport-Nielsen[2], E. Chowdhury[1], J. Sartin[1], E. Reid[2], B. Kaltenboeck[1]; 1Auburn University, Auburn, AL, USA, 2Barrington Dairy, Montezuma, GA, USA.
10:30	**006**	Genome scanning for conditionally essential genes in *Salmonella.*	**A. Khatiwara**, T. Jiang, T. Dawoud, Y. Kwon; University of Arkansas, Fayetteville, AR, USA.
10:45-11:30 Keynote	**007**	Exploring APEC virulence, evolution, and host specificity using pathogenomic approaches.	**L.K. Nolan**; Iowa State University, Ames, IA, USA.
11:30		**Lunch Break**	
		Presiders: Jun Lin and Orhan Sahin	
1:30 Mon.	**008**	Molecular characterization of Egyptian *Escherichia coli* strains isolated from broiler chickens and their environment.	**A.H. Hussein**[1], I.A. Ghanem[1], A.A. Mahdi[1], C.M. Logue[2], L.K. Nolan[2]; 1Department of Avian and Rabbit Medicine, Faculty of Veterinary Medicine, Zagazig University, Zagazig, Egypt, 2Department of Veterinary Microbiology and Preventive Medicine, College of Veterinary Medicine, Iowa State University, Ames, IA, USA.
1:45	**009**	The siderophore esterases Fes and IroD contribute to virulence of avian extra-intestinal pathogenic *Escherichia coli.*	M. Caza, F. Lepine, **C.M. Dozois**; INRS-Institut Armand-Frappier, Laval, QC, Canada.
2:00	**010**	Genetic factors affecting the persistence and dissemination of *bla* $_{CMY-2}$ positive IncA/C plasmids.	**K.S. Lang**, J.L. Danzeisen, P. Holtegaard, T.J. Johnson; Dept. of Veterinary and Biomedical Sciences, University of Minnesota, Saint Paul, MN, USA.

(continued)

BACTERIAL PATHOGENESIS

Avenue Ballroom - 4th Floor

Section Leader: Gireesh Rajashekara

Time	No.	Title	Authors
2:15 Mon.	**011**	The Cia proteins of Campylobacter jejuni are delivered to the cytosol of host cells via a flagellar-dependent mechanism.	**J. Neal-McKinney**, M. Konkel; Washington State University, Pullman, WA, USA.
2:30	**012**	Characterization of the methyl accepting chemotaxis proteins in *Campylobacter jejuni* .	**K. Chandrashekhar***, D. Gangaiah, G. Rajashekara; The Ohio State University, Wooster, OH, USA.
2:45		**Break and Table Top Exhibits – Foyer**	
		Presiders: Orhan Sahin and Gireesh Rajashekara	
3:00	**013**	*In vitro* characterization of equine source hyperimmune plasma against *Bacillus anthracis* toxins.	**M. Caldwell**, K.V. Brock; Auburn University, Auburn, AL, USA.
3:15	**014**	Edwardsiella ictaluri cyclic adenosine 3', 5'-monophosphate receptor protein (crp) mutant vaccine and boost immunization in catfish (Ictalurus punctatus).	**J. Santander**, R.I. Curtiss; Arizona State University, Tempe, AZ, USA.
3:30	**015**	Identification of molecular targets for diagnosis of bovine tuberculosis.	**A.L. Lim**, J.P. Steibel, S.R. Bolin; Michigan State University, E. Lansing, MI, USA.
3:45	**016**	Genomic signatures of M paratuberculosis from multiple animal species: a better understanding of Johne's disease transmission.	P. Ghosh[1], M. Shehata[2], E. Alyamani[2], M. Eldabib[3], M. Hashad[3], A. Alnaeem[4], K. Busadah[4], A. Alswailem[2], **A.M. Talaat**[1]; 1University of Wisconsin-Madison, Madison, WI, USA, 2King Abdulaziz City of Science and Technology, Ryiadh, Saudi Arabia, 3Qassim University, Qassim, Saudi Arabia, 4King Faisal University, Al-hassa, Saudi Arabia.
4:00 Mon.	**017**	*Mycobacterium avium* subspecies paratuberculosis produces spores.	**E. Lamont**[1], J.P. Bannantine[2], A. Armien[1], D. Ariyakumar[1], S. Sreevatsan[1]; 1University of Minnesota, Saint Paul, MN, USA, 2National Animal Disease Center, United States Department of Agriculture, Ames, IA, USA.
4:15	**018**	Molecular characterization of the Mycobacteria antigen 85 complex and Fibronectin interaction.	C.-J. Kuo, **Y.-F. Chang**; Cornell. University, Ithaca, NY, USA.
4:30 to	**5:00**	**Break and Table Top Exhibits – Foyer**	
5:00 to	**6:30**	**Poster Session II Grand Ballroom Salon III - 7th floor**	

(continued)

BACTERIAL PATHOGENESIS

Avenue Ballroom - 4th Floor

Section Leader: Gireesh Rajashekara

Time	No.	Title	Authors
		Presiders: Gireesh Rajashekara and Jun Lin	
8:00 Tues.	**019**	The structure of a calcium-binding leptospiral immunoglobulin-like protein (LigB) domain of Leptospira interrogans.	C.P. Ptak[1], C.-L. Hsieh[1], Y.-P. Lin[1], A.S. Maltsev[2], R. Raman[3], Y. Sharma[3], **Y.-F. Chang**[1]; 1Cornell. University, Ithaca, NY, USA, 2National Institutes of Health, Bethesda,, Bethesda, MD, USA, 3Center for Cellular and Molecular Biology, Hyderabad, India.
8:15	**020**	Evaluation of oral fluid samples as a diagnostic sample for detection of *Erysipelothrix rhusiopathiae* in pigs.	**L.G.G. Lirola, Jr.**, P.G. Halbur, T. Opriessnig; ISU, Ames, IA, USA.
8:30	**021**	Seroprevalence of brucellosis in sheep in the selected areas of Mymensingh and Netrakona districts of Bangladesh.	**M.S. Rahman**, M. Ahsan, M. Rahman, N. Jahan; Bangladesh Agricultural University, Mymensingh, Bangladesh.
8:45	**022**	Identification of antigens for more reliable *Streptococcus equi* specific ELISA assay.	**S. Artiushin**, S. Velineni, J.F. Timoney; University of Kentucky, Lexington, KY, USA.
9:00	**023**	The role of Th17 cytokines, IL-17A and IL-22, during Listeria monocytogenes infection of the pregnant mouse.	**K.P. Poulsen**, N.G. Faith, H. Steinberg, C.J. Czuprynski; University of Wisconsin-Madison, Madison, WI, USA.
9:15	**024**	Diversity among clostridial isolates from cellulitis cases in turkeys.	**A.J. Thachil**, K.V. Nagaraja; Department of Veterinary and Biomedical Sciences, University of Minnesota, Saint Paul, MN, USA.
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**	

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

Time	No.	Title	Authors
11:30		**Lunch Break**	
1:30 Mon.	**025**	Biosecurity assessment as a tool towards risk-based surveillance on swine farms in southern Ontario.	**K. Bottoms**, Z. Poljak, C. Dewey, R. Friendship; University of Guelph, Guelph, ON, Canada.
1:45	**026**	Evaluation study of interventions for reducing the risk of PRRSV introduction into filtered farms via retrograde air movement (Back-drafting) through idle fans.	**C. Alonso Garcia-Mochales**[1], S. Otake[1], P. Davies[1], S. Dee[2]; 1University of Minnesota, St. Paul, MN, USA, 2Pipestone Veterinary Clinic, Pipestone, MN, USA.
2:00	**027**	Isolation of Salmonella organisms from the environment in a large animal hospital using electrostatic (Swiffer) and sterile sponge collection devices.	**A. Ruple**, B. Burgess, P.S. Morley; Colorado State University, Fort Collins, CO, USA.
2:15	**028**	A novel animal product-free avian cell line that supports high titer influenza virus replication.	**M. Carvajal-Yepes**, K.A. Smith, T. Almy, C.J. Colvin, P.M. Coussens; Michigan State University, East Lansing, MI, USA.
2:30 Mon.	**029**	Development of multiplex-PCR and electronic microarrays for detection and typing of avian and bovine high consequence viruses.	O. Lung[1], D. Deregt[1], D. Hodko[2], J. Pasick[3], Z. Zhang[3], D. King[4], S. Reid[4], K. Burton Hughes[1], T. Furukawa-Stoffer[1], S. Ohene-Adjei[1], A. Beeston[1], M. Fisher[1], K. Hahn[1], **A. Ambagala**[1]; 1Canadian Food Inspection Agency, Lethbridge, AB, Canada, 2Nexogen, Inc., San Diego, CA, USA, 3Canadian Food Inspection Agency, Winnipeg, MB, Canada, 4Institute for Animal Health, Pirbright, Surrey, UK.
2:45		**Break and Table Top Exhibits – Foyer**	
3:00-3:45 Keynote	**030**	Working with Biolevel 3 Agents that interface across human, livestock, and wildlife boundaries.	**S. Olsen,** Infectious Bacterial Diseases of Livestock Research Unit, National Animal Disease Center, Ames, IA.
4:30 to	**5:00**	**Break and Table Top Exhibits – Foyer**	
5:00 to	**6:30**	**Poster Session II Grand Ballroom Salon III - 7th floor**	

COMPANION ANIMAL EPIDEMIOLOGY

Denver/Houston Room - 5th Floor

Section Leader: Margaret Slater and Laura Hungerford

Time	No.	Title	Authors
		Presiders: Margaret Slater and John New	
8:00-8:45 Tues. Keynote	**063**	**Keynote presentation is in the Epidemiology Section, Salons A/B/C/D Room, 5th Floor:** Epidemiologists: we're not the same as statisticians.	**A. O'Connor**; Iowa State University, Ames, IA, USA.
8:45	**031**	Prevalence and risk factors for shedding of *Campylobacter* spp. in dogs that frequent dog parks in southern Ontario, Canada.	**T.D. Procter**[1], D.L. Pearl[2], R.L. Finley[3], E.K. Leonard[2], N. Janecko[2], J.S. Weese[4], A.S. Peregrine[4], R.J. Reid-Smith[5], J.M. Sargeant[1]; 1Department of Population Medicine, and Centre for Public Healh and Zoonoses, Ontario Veterinary College, University of Guelph, Guelph, ON, Canada, 2Department of Population Medicine, Ontario Veterinary College, University of Guelph, Guelph, ON, Canada, 3Centre for Food-borne, Environmental and Zoonotic Infectious Diseases, Public Health Agency of Canada, Guelph, ON, Canada, 4Department of Pathobiology, Ontario Veterinary College, University of Guelph, Guelph, ON, Canada, 5Laboratory of Foodborne Zoonoses, Public Health Agency of Canada, Guelph, ON, Canada
9:00	**032**	Comparison of virus isolation, one-step real-time reverse transcriptase-PCR assay, and two rapid influenza diagnostic tests for detecting canine influenza virus (H3N8) shedding in dogs.	**H.L. Pecoraro**, M.E. Spindel, S. Bennett, K.F. Lunn, G.A. Landolt; Colorado State University, Fort Collins, CO, USA.
9:15	**033**	Comparison of the geographical distribution of Feline Leukemia virus (FeLV) and Feline Immunodeficiency virus (FIV) infections in the United States (2000-2011).	**B. Chhetri**, O. Berke, D.L. Pearl, D. Bienzle; University of Guelph, Guelph, ON, Canada.
9:30		**Break and Table Top Exhibits – Foyer**	
10:00	**034**	Discussion: Help shape the future of companion animal epidemiology at CRWAD	**M. Slater1,** The American Society for the Prevention of Cruelty to Animals, Northampton, MA, USA

(continued)

COMPANION ANIMAL EPIDEMIOLOGY

Denver/Houston Room - 5th Floor

Section Leader: Margaret Slater and Laura Hungerford

Time	No.	Title	Authors
10:15 Tues.	**035**	Prevalence of and exposure factors for H3N8 canine influenza virus seropositivity in US dogs with influenza-like illness.	**T.C. Anderson**[1], P.C. Crawford[1], E.J. Dubovi[2], E.P.J. Gibbs[3], J.A. Hernandez[4]; 1Department of Small Animal Clinical Sciences, College of Veterinary Medicine, University of Florida, Gainesville, FL, USA, 2Animal Health Diagnostic Center, College of Veterinary Medicine, Cornell University, Ithaca, NY, USA, 3Department of Infectious Diseases and Pathology, College of Veterinary Medicine, University of Florida, Gainesville, FL, USA, 4Department of Large Animal Clinical Sciences, College of Veterinary Medicine, University of Florida, Gainesville, FL, USA.
10:30	**036**	Effect of Skin lessions on Haematological Picture of some Dogs in Ibadan.	**I.A. Adetiba**; University of Ibadan, Nigeria, Ibadan, Nigeria.
10:45	**037**	Modeling feral cat population dynamics in Knox County, TN.	L.E. Lee[1], A.T.N. Nguyen[1], S.M. Lenhart[1], N. Robl[2], A.M. Bugman[3], **J.C. New, Jr.**[4], B. Lammers[4], T.L. Jennings[4], H. Weimer[5]; 1University of Tennessee Department of Mathematics, Knoxville, TN, USA, 2University of Wisconsin College of Veterinary Medicine, Madison, WI, USA, 3University of Illinois College of Veterinary Medicine, Urbana, IL, USA, 4University of Tennessee College of Veterinary Medicine, Knoxville, TN, USA, 5Private consultant, Knoxville, TN, USA
11:00	**038**	Using logistic regression models to create a scoring system for socialization status of shelter cats during a three day holding period.	**M. Slater**[1], K.A. Miller[2], E. Weiss[3], A. Mirontschuk[4], K.V. Makolinski[5]; 1The American Society for the Prevention of Cruelty to Animals, Northampton, MA, USA, 2The American Society for the Prevention of Cruelty to Animals, New York, NY, USA, 3The American Society for the Prevention of Cruelty to Animals, Benton, KS, USA, 4The American Society for the Prevention of Cruelty to Animals, Oakland, CA, USA, 5The American Society for the Prevention of Cruelty to Animals, Orchard Park, NY, USA.

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COMPANION ANIMAL EPIDEMIOLOGY

Denver/Houston Room - 5th Floor

Section Leader: Margaret Slater and Laura Hungerford

Time	No.	Title	Authors
11:15 Tues.	039	Development and implementation of a Shelter Population Index (SPI) to evaluate population trends of cats and dogs in the United States.	M. Gruen[1], **J.C. New, Jr.**[2], R. Ruch-Gallie[3], M.C. Antognoli[4], T.L. Jennings[2], M. Boden[5], M. Salman[3], S.L. Zawistowski[6], M.R. Slater[7], D.D. Dunning[1]; 1North Carolina State University College of Veterinary Medicine, Raleigh, NC, USA, 2University of Tennessee College of Veterinary Medicine, Knoxville, TN, USA, 3Colorado State University College of Veterinary Medicine and Biomedical Sciences, Ft. Collins, CO, USA, 4USDA, APHIS, VS, Ft. Collins, CO, USA, 5Tampa Bay Society for the Prevention of Cruelty to Animals, Tampa, FL, USA, 6American Society for the Prevention of Cruelty to Animals, New York, NY, USA, 7American Society for the Prevention of Cruelty to Animals, Urbana, IL, USA.
11:45 to	**12:30**	**Business Meeting, Dedication, New Members Introduction, and Graduate Student Competition Awards Presentations**	

EPIDEMIOLOGY AND ANIMAL HEALTH ECONOMICS

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

Section Leader: Ashley Hill

Time	No.	Title	Authors
8:00 Mon.	**040**	Investigation of pig mortalities in Maha Sarakham Province, Thailand, October 2010.	**S. Pisek**[1], P. Srisai[1], K. Thammasar[2], D. Singpan[3], P. Cheewajorn[3], K. Wongsathapornchai[4], C. Jiraphongsa[5]; 1Field Epidemiology Training Program for Veterinarian, Bangkok, Thailand, 2Nongkhai Provincial Livestock Office, Nongkhai, Thailand, 3Maha Sarakham Provincial Livestock Office, Maha Sarakham, Thailand, 4Regional Office for Asia and the Pacific, Food and Agriculture Organization of the United Nations (FAO), Bangkok, Thailand, 5Field Epidemiology Training Program (FETP), Bangkok, Thailand.
8:15	**041**	Adjusting disease freedom confidence for imperfect diagnostic accuracy: A review of the evidence for non-traditional diagnostic specimens tested for PRRSV.	**L. Rosengren**[1], Z. Poljak[2], C.A. Gagnon[3]; 1Rosengren Epidemiology Consulting Ltd., Midale, SK, Canada, 2Dept. of Population Medicine, Ontario Veterinary College, University of Guelph, Guelph, ON, Canada, 3Groupe de recherche sur les maladies infectieuses du porc (GREMIP), Faculte de medecine veterinaire, Universite de Montreal, Montreal, QC, Canada.
8:30	**042**	Modeling of porcine reproductive and respiratory syndrome virus infection in a pig herd.	**H. Le**, Z. Poljak, R. Deardon, C. Dewey; University of Guelph, Guelph, ON, Canada.
8:45	**043**	Baseline study in the Niagara region porcine reproductive and respiratory syndrome (PRRS) area regional control and elimination project (ARC&E).	**Z. Poljak**[1], J. Carpenter[2], M. Misener[3], G. Charbonneau[3], B. Jones[3], J. Fairles[4], J. Alsop[5]; 1Department of Population Medicine, University of Guelph, Guelph, ON, Canada, 2OPIC OSHAB, Stratford, ON, Canada, 3South West Ontario Veterinary Services, Stratford, ON, Canada, 4Animal Health Laboratory, University of Guelph, Guelph, ON, Canada, 5Ontario Ministry of Agriculture, Food, and Rural Affairs, Guelph, ON, Canada.
9:00	**044**	Association between PRRSV ORF5 genetic distance and differences in space, time, ownership, and animal sources among commercial pig herds.	**T. Rosendal**, C. Dewey, R. Friendship, S. Wootton, B. Young, Z. Poljak; University of Guelph, Guelph, ON, Canada.
9:15	**045**	The association between the PCVAD outbreak in Ontario and the positivity of Porcine Reproductive and Respiratory Syndrome virus ELISA and PCR test results.	**T. O'Sullivan**[1], R. Friendship[1], D. Pearl[1], B. McEwen[2], C. Dewey[1]; 1University of Guelph, Guelph, ON, Canada, 2Animal Health Laboratory, University of Guelph, Guelph, ON, Canada.
9:30		**Break and Table Top Exhibits – Foyer**	

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EPIDEMIOLOGY AND ANIMAL HEALTH ECONOMICS

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

Section Leader: Ashley Hill

Time	No.	Title	Authors
		Presiders: Paivi Rajala-Schultz and Sara Gragg	
10:00 Mon.	**046**	The impact of maternally derived immunity on influenza virus transmission in neonatal pig populations.	**M. Allerson**, A. Romagosa, J. Deen, M. Gramer, H. Joo, M. Torremorell; University of Minnesota, St. Paul, MN, USA.
10:15	**047**	Sensitivity of oral fluids for the detection of influenza virus in young pig populations with and without maternally derived immunity.	**M. Allerson**, M. Torremorell; University of Minnesota, St. Paul, MN, USA.
10:30	**048**	Detection of influenza A virus in aerosols from acutely infected pig populations.	**C. Corzo**, M. Torremorell, M. Gramer, R. Morrison; University of Minnesota, Saint Paul, MN, USA.
10:45	**049**	Influenza A virus genetic diversity in immune pigs.	**C.A. Diaz**, M.W. Allerson, A. Romagosa, G.R. Marie, S. Sreevatsan, M. Torremorell; University of Minnesota, Saint Paul, MN, USA.
11:00	**050**	Estimating hiding behavior for lameness in sows.	**N. Homwong**, J. Deen, S.K. Baidoo; University of Minnesota, St Paul, MN, USA.
11:15	**051**	The effect of lameness and other morbidity causes on average daily gain in feedlot cattle.	**G.T. Kruse**[1], R.F. Randle[1], D.E. Hostetler[1], G.K. Tibbetts[2], D. Griffin[3], D.R. Smith[1]; 1University of Nebraska-Lincoln, Lincoln, NE, USA, 2Zinpro Corporation, Eden Prairie, MN, USA, 3University of Nebraska-Lincoln, Clay Center, NE, USA
11:30		**Lunch Break**	
		Presiders: Annette O'Connor and Katharine Benedict	
1:30 Mon.	**052**	Risk factors for environmental contamination with *Salmonella enterica* in a veterinary teaching hospital.	**B.A. Burgess**, P.S. Morley; Colorado State University, Fort Collins, CO, USA.
1:45	**053**	*Salmonella* shedding in hospitalized horses with signs of colic, with or without diarrhea.	**A. Ekiri**, A. Morton, M. Long, T. Krueger, R. MacKay, J. Hernandez; University of Florida, Gainesville, FL, USA.
2:00	**054**	Management practices associated with *Salmonella* or antimicrobial resistant *Salmonella* on United States dairy herds.	**G. Habing**[1], J. Lombard[2], C. Kopral[2], D. Dargatz[2], J.B. Kaneene[1]; 1Center for Comparative Epidemiology, College of Veterinary Medicine, Michigan State University, East Lansing, MI, USA, 2Centers for Epidemiology and Animal Health, Veterinary Services, Animal Plant and Health Inspection Service, United States Department of Agriculture, Fort Collins, CO, USA
2:15	**055**	Phenotypic and genotypic characterization of methicillin-resistant *Staphylococcus pseudintermedius* in dogs, cats, and horses at a veterinary teaching hospital.	**J. Mathews**[1], N. Tiao[1], P. Patchanee[2], W. Gebreyes[1]; 1The Ohio State University, Columbus, OH, USA, 2Chiang Mai University, Chiang Mai, Thailand.

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EPIDEMIOLOGY AND ANIMAL HEALTH ECONOMICS

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

Section Leader: Ashley Hill

Time	No.	Title	Authors
2:30 Mon.	**056**	Phenotypic and genotypic characterization of Methicillin-resistant *Staphylococcus aureus* in dogs, cats, and horses at a veterinary teaching hospital from 2007 to 2010.	**J. Mathews**[1], N. Tiao[1], P. Patchanee[2], W. Gebreyes[1]; 1The Ohio State University, Columbus, OH, USA, 2Chiang Mai University, Chiang Mai, Thailand.
2:45		**Break and Table Top Exhibits – Foyer**	
		Presiders: Guy Loneragan and Brandy Burgess	
3:00	**057**	Using agent-based modeling of the village poultry sector in Thailand to identify opportunities for influenza transmission and potential interventions for disease control.	**A.L. Beaudoin**[1], R. Singer[1], J. Bender[1], A. Isaac[2]; 1Department of Population Medicine University of Minnesota, Saint Paul, MN, USA, 2Department of Economics American University, Washington D.C., DC, USA.
3:15	**058**	Hierarchical Bayesian modeling of heteroskedasticity in average daily weight gain of feedlot cattle.	**S. Xiang**[1], D. Renter[2], N. Cernicchiaro[2], B. White[3], N.M. Bello[4]; 1Department of Statistics, College of Arts and Sciences, Kansas State University, Manhattan, KS, USA, 2Department of Diagnostic Medicine/Pathobiology, College of Veterinary Medicine, Kansas State University, Manhattan, KS, USA, 3Department of Clinical Sciences, College of Veterinary Medicine, Kansas State University, Manhattan, KS, USA, 4Department of Statistics, College of Arts and Sciences, Department of Diagnostic Medicine/Pathobiology, College of Veterinary Medicine, Kansas State University, Manhattan, KS, USA.
3:30	**059**	Culling decisions based on microbiological and serological test results for the control of Johne's disease in beef cow-calf operations.	**B. Bhattarai**[1], G.T. Fosgate[2], J.B. Osterstock[3], S.C. Park[4], A.J. Roussel[5]; 1Department of Veterinary Integrative Biosciences, Texas A&M University, College Station, TX, USA, 2Department of Production Animal Studies, University of Pretoria, Onderstrpoort, South Africa, 3Feedlot Decision Support / Pfizer Animal Genetics, Kalamazoo, MI, USA, 4Texas AgriLife Research and Extension Center, Vernon, TX, USA, 5Department of Veterinary Large Animal Clinical Sciences, Texas A&M University, College Station, TX, USA.

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EPIDEMIOLOGY AND ANIMAL HEALTH ECONOMICS

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

Section Leader: Ashley Hill

Time	No.	Title	Authors
3:45 Mon.	**060**	Associations of antimicrobial use and antimicrobial resistance in *Escherichia coli* isolates individually sampled from feedlot cattle.	**K. Benedict**[1], S. Gow[2], S. Checkley[3], C. Booker[4], S. Hannon[4], T. McAllister[5], P. Morley[1], R. Reid-Smith[6]; 1Colorado State University, Fort Collins, CO, USA, 2Public Health Agency of Canada, Saskatoon, SK, Canada, 3University of Calgary, Calgary, AB, Canada, 4Feedlot Health Management Services, Okotoks, AB, Canada, 5University of Lethbridge, Lethbridge, AB, Canada, 6Public Health Agency of Canada, Guelph, ON, Canada.
4:00	**061**	Effect of intervention strategies on ceftiofur resistance determinant (blaCMY-2 gene) and its relationship with TetA and TetB genes in cattle.	**N. Kanwar**[1], H.M. Scott[1], B. Norby[2], S. Moore[3], J. Vinasco[1], G.H. Loneragan[4], M.M. Chengappa[1], J. Bai[1]; 1Kansas State University, Manhattan, KS, USA, 2Michigan State University, East Lansing, MI, USA, 3West Texas A&M University, Canyon, TX, USA, 4Texas Tech University, Lubbock, TX, USA.
4:15	**062**	Genotypic characterization and comparison of tetracycline-resistant *Escherichia coli* isolates arising from humans and swine in a vertically integrated agri-food system.	**G.E. Agga**, M.H. Scott, J. Vinasco; Kansas State University, Manhattan, KS, USA.
4:30 to	**5:00**	**Break and Table Top Exhibits – Foyer**	
5:00 to	**6:30**	**Poster Session II Grand Ballroom Salon III - 7th floor**	

EPIDEMIOLOGY AND ANIMAL HEALTH ECONOMICS

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

Section Leader: Ashley Hill

Time	No.	Title	Authors
		Presiders: Ashley Hill	
8:00-8:45 Tues. Keynote	**063**	Epidemiologists: we're not the same as statisticians.	**A. O'Connor**; Iowa State University, Ames, IA, USA.
8:45	**064**	Understanding geographic epidemiology: the geographic epidemiologic trillium.	**O. Berke**; University of Guelph, Guelph, ON, Canada.
9:00	**065**	Methodological comparisons for antimicrobial resistance surveillance in feedlot cattle.	**K. Benedict**[1], S. Gow[2], S. Checkley[3], C. Booker[4], T. McAllister[5], P. Morley[1], R. Reid-Smith[6]; 1Colorado State University, Fort Collins, CO, USA, 2Public Health Agency of Canada, Saskatoon, SK, Canada, 3University of Calgary, Calgary, AB, Canada, 4Feedlot Health Management Services, Okotoks, AB, Canada, 5University of Lethbridge, Lethbridge, AB, Canada, 6Public Health Agency of Canada, Guelph, ON, Canada
9:15	**066**	Multi-drug resistance in Ontario swine *Streptococcus suis, Escherichia coli* K88, and *Pasteurella multocida* isolates (1998 - 2010).	**S. Glass-Kaastra**[1], D.L. Pearl[2], J. Parmley[3], R. Reid-Smith[4], D. Leger[3], A. Agunos[3], B. McEwen[5], D. Slavic[5], S.A. McEwen[2], J. Fairles[5]; 1Population Medicine, University of Guelph, Guelph, ON, Canada, 2Department of Population Medicine, University of Guelph, Guelph, ON, Canada, 3Public Health Agency of Canada, Guelph, ON, Canada, 4Public Health Agency of Canada; Department of Population Medicine, University of Guelph; Animal Health Laboratory, University of Guelph, Guelph, ON, Canada, 5Animal Health Laboratory, University of Guelph, Guelph, ON, Canada.
9:30		**Break and Table Top Exhibits – Foyer**	
		Evan Chaney	
10:00 Tues.	**067**	Effect of bovine Corona virus shedding and seropositivity on the risk for BRD in calves transitioning from the farm of origin to the feedlot.	**A. O'Connor**, T. Engelken, V. Cooper, R. Dewell, P. Plummer; Iowa State University, Ames, IA, USA.
10:15	**068**	Q-fever in small ruminants in Indiana.	**A. Johnson**[1], J. Mungin[2], R. Pogranichniy[1], K. Thakur[1], C. Miller[3], R. Vemulapalli[1]; 1Purdue University, West Lafayette, IN, USA, 2Tuskegee University, Tuskegee, AL, USA, 3Indiana State Board of Animal Health, Indianapolis, IN, USA.
10:30	**069**	Seroprevalence of brucellosis and its association with other reproductive diseases in buffaloes in Bangladesh.	**M.S. Rahman**; Bangladesh Agricultural University, Mymensingh, Bangladesh.

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EPIDEMIOLOGY AND ANIMAL HEALTH ECONOMICS

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

Section Leader: Ashley Hill

Time	No.	Title	Authors
10:45 Tues.	**070**	Prevalence of *Toxoplasma gondii* in market age lambs in the United States.	**C.R. Kristensen**[1], O. Kwok[2], K. Marshall[1], J. Dubey[2]; 1USDA-APHIS, Fort Collins, CO, USA, 2USDA-ARS, Beltsville, MD, USA.
11:00	**071**	Prevalence of elevated temperatures among horses presented for importation to the United States.	**J. Traub-Dargatz**[1], B. Bischoff[2], C. Kopral[2], J. Rodriguez[2]; 1Colorado State University, Fort Collins, CO, USA, 2USDA:APHIS:VS Centers for Epidemiology and Animal Health, Fort Collins, CO, USA.
11:45 to	**12:30**	**Business Meeting, Dedication, New Members Introduction, and Graduate Student Competition Awards Presentations**	

FOOD AND ENVIRONMENTAL SAFETY

Salon E - 5th Floor

Section Leader: Yvette Johnson-Walker

Time	No.	Title	Authors
		Presiders: Charles Thoen and Tracey Nicholson	
8:00 Mon.	**072**	Piloting the future: results from a pilot study for changes in the animal sampling program for the national antibiotic resistance monitoring system (narms).	**M. Torrence**[1], R. Singer[2]; 1USDA, ARS, Kearneysville, WV, USA, 2University of MN, St. Paul, MN, USA.
8:15	**073**	Prevalence and antimicrobial resistance of potential food safety pathogens on united states beef cow-calf operations	**D. Dargatz**; USDA:APHIS CEAH, Fort Collins, CO, USA.
8:30	**074**	The use of multi-level model residuals for food animal disease surveillance.	**G.D. Alton**[1], D.L. Pearl[1], K.G. Bateman[1], W.B. McNab[2], O. Berke[1]; 1University of Guelph, Guelph, ON, Canada, 2Ontario Ministry of Agriculture, Food and Rural Affairs, Guelph, ON, Canada.
8:45	**075**	Vaccination to control *Escherichia coli* O157 in integrated cattle production systems.	**G.H. Loneragan**[1], D.H. Thomson[2], B.A. Butler[2], M.M. Brashears[1], R.M. McCarthy[1], T.M. Arthur[3], J.M. Bosilevac[3], N. Kalchayanand[3], J.W. Schmidt[3], T.L. Wheeler[3], A.L. Siemens[4], D.L. Schaefer[4], C.B. Rose[4], J.R. Ruby[5], T.C. Bryant[5], R.J. Algino[5], B.W. Wileman[6], D.T. Burkhardt[6], L.M. Slinden[6], D.A. Emery[6]; 1Texas Tech University, Lubbock, TX, USA, 2Kansas State University, Manhattan, KS, USA, 3USMARC, ARS, USDA, Clay Center, NE, USA, 4Cargill Meat Solutions, Wichita, KS, USA, 5JBS USA, Greeley, CO, USA, 6Epitopix LLC, Willmar, MN, USA.
9:00	**076**	Prevalence of Shiga toxin-producing Escherichia coli (STEC) genes by multiplex PCR in cattle and their environment, Michigan 2011.	**C. Venegas-Vargas**, J. Zingsheim, T. Neuman, R. Mosci, L. Ouellette, A. Khare, P. Singh, S. Rust, P. Bartlett, S. Manning, D. Grooms; Michigan State University, East Lansing, MI, USA.
9:15	**077**	Multiple-locus variable-nucleotide tandem repeat analysis of *Escherichia coli* O157:H7 evaluating isolate distribution on a closed feedlot facility, Wooster, OH.	**M. Williams**[1], D. Pearl[2], J. LeJeune[1]; 1Ohio State University, Wooster, OH, USA, 2University of Guelph, Guelph, ON, Canada.
9:30		**Break and Table Top Exhibits – Foyer**	
		Presiders: Nicol Janecko	
10:00 Mon.	**078**	Intimin type characterization of non-O157 Shiga toxin-producing *Escherichia coli* isolates.	Z.R. Stromberg, M.M. Hille, G.L. Lewis, **R.A. Moxley**; University of Nebraska-Lincoln, Lincoln, NE, USA.
10:15	**079**	Multistate Markov chain model to describe and compare fecal shedding dynamics of three *Escherichia coli* O157:H7 strains in cattle.	**R. Gautam**[1], D. Dopfer[2], C.W. Kaspar[2], M. Kulow[2], T.K. Gonzales[2], K.M. Pertzborn[2], R.J. Carroll[1], W.E. Grant[1], R. Ivanek[1]; 1Texas A&M, College Station, TX, USA, 2University of Wisconsin, Madison, WI, USA.

FOOD AND ENVIRONMENTAL SAFETY

Salon E - 5th Floor

Section Leader: Yvette Johnson-Walker

Time	No.	Title	Authors
10:30 Mon.	**080**	Characterization of *Escherichia coli* carrying *bla* $_{CTX-M}$ isolated from fecal flora of dairy cattle.	**D.F. Mollenkopf**; Ohio State University, Columbus, OH, USA.
10:45	**081**	Validation of culture methods for non-O157 Shiga toxin-producing *Escherichia coli.*	G.L. Lewis, **R.A. Moxley**; University of Nebraska-Lincoln, Lincoln, NE, USA.
11:00	**082**	Simulation model of vaccinating cattle against STEC O157 for pre-harvest food safety.	**A.R. Vogstad**, R.A. Moxley, G.E. Erickson, T.J. Klopfenstein, D.R. Smith; University of Nebraska-Lincoln, Lincoln, NE, USA.
11:15	**083**	Virulence profiling of Shiga toxin-producing *Escherichia coli* O111:NM isolates from cattle.	**M. Karama**; Western University of Health Sciences, Pomona, CA, USA.
11:30		**Lunch Break**	
		Presiders: Julie Funk and Alda Pires	
1:30 Mon.	**084**	Meta-analysis of a three-dose regimen of a type III secreted protein vaccine for efficacy at reducing STEC O157 in feces of feedlot cattle.	**A.R. Vogstad**[1], R.A. Moxley[1], G.E. Erickson[1], T.J. Klopfenstein[1], D. Rogan[2], R. Culbert[2], D.R. Smith[1]; 1University of Nebraska-Lincoln, Lincoln, NE, USA, 2Bioniche Life Sciences, Belleville, ON, Canada.
1:45	**085**	Antimicrobial resistance in *Escherichia coli* recovered from feedlot cattle.	**N. Noyes**[1], K. Benedict[1], S. Gow[2], C. Booker[3], T. McAllister[4], R. Reid-Smith[5], S. Hannon[3], P. Morley[1]; 1Colorado State University, Fort Collins, CO, USA, 2Public Health Agency of Canada, Saskatoon, SK, Canada, 3Feedlot Health Management Services, Okotoks, AB, Canada, 4University of Lethbridge, Lethbridge, AB, Canada, 5Public Health Agency of Canada, Guelph, ON, Canada.
2:00	**086**	Effects of a vaccine and a direct-fed microbial on fecal shedding of *E. coli* O157:H7 in pens of commercial feedlot cattle fed a diet supplemented with distiller's grains.	**C.A. Cull**[1], D.G. Renter[1], Z.D. Paddock[1], N.M. Bello[1], A.H. Babcock[2], T.G. Nagaraja[1]; 1Kansas State University, Manhattan, KS, USA, 2Adam's Land and Cattle Company, Broken Bow, NE, USA.
2:15	**087**	Fecal shedding of *Escherichia coli* O26 in feedlot cattle from a field trial evaluating an *Escherichia coli* O157:H7 vaccine and a direct-fed microbial.	**Z.D. Paddock**, D.G. Renter, C.A. Cull, L.A. Schaefer, X. Shi, S. Li, J. Bai, T. Nagaraja; Kansas State University, Manhattan, KS, USA.
2:30	**088**	Development of a semi-quantitative ranking scheme to estimate the concentration of *Escherichia coli* O157:H7 in bovine feces.	**W.E. Chaney**[1], G.H. Loneragan[1], M. Scott[2], M.M. Brashears[1]; 1Texas Tech University, Lubbock, TX, USA, 2Kansas State University, Manhattan, KS, USA.
2:45		**Break and Table Top Exhibits – Foyer**	

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FOOD AND ENVIRONMENTAL SAFETY

Salon E - 5th Floor

Section Leader: Yvette Johnson-Walker

Time	No.	Title	Authors
		Presiders: Yvette Johnson	
3:00 Mon.	**089**	Modeling the effect of bacterial transfer rates and interventions on the prevalence and concentration of *Escherichia coli* O157 on beef carcasses.	**M. Jacob**[1], M. Sanderson[2], C. Dodd[3], D. Renter[2]; 1North Carolina State University, Raleigh, NC, USA, 2Kansas State University, Manhattan, KS, USA, 3U. S. Army Public Health Command Region- Europe, Landstuhl, Germany
3:15	**090**	Assessing risks of microbial contamination of produce from irrigation water.	**G. Won**, J. LeJeune; The Ohio State University, Wooster, OH, USA.
3:30	**091**	Inactivation kinetic of feline calicivirus (norovirus surrogate) on lettuce by electron beam irradiation.	**F. Zhou**, J. Dickson, K.M. Harmon, O.G. Dennis, K.-J. Yoon, S. Neibuhr; Iowa State University, Ames, IA, USA.
3:45	**092**	Effects of feeding copper sulfate, tetracycline and tylosin on the prevalence of transferable copper resistance gene, *tcrB* , among fecal enterococci of swine.	**R.G. Amachawadi**, H.M. Scott, N.W. Shelton, M.D. Tokach, J. Vinasco, T.R. Mainini, S.S. Dritz, J.L. Nelssen, T. Nagaraja; Kansas State University, Manhattan, KS, USA.
4:00 Mon.	**093**	Export risk assessment for the export of deboned beef from South Africa to Egypt.	**J.W. Oguttu**[1], E.M. Midzi[2], S. Ramrajh[3]; 1University of South Africa, Pretoria, South Africa, 2South African Veterinary Services, National Dept. of Agriculture, Mafikeng, Northwest, South Africa, 3South African Veterinary services, National Dept. of Agriculture, Durban, Kwazulu-Natal, South Africa.
4:30 to	**5:00**	**Break and Table Top Exhibits – Foyer**	
5:00 to	**6:30**	**Poster Session II Grand Ballroom Salon III - 7th floor**	

FOOD AND ENVIRONMENTAL SAFETY

Salon E - 5th Floor

Section Leader: Yvette Johnson-Walker

Time	No.	Title	Authors
		Presiders: Michele Williams	
8:00-8:45 Tues. Keynote	**063**	**Keynote presentation is in the Epidemiology Section, Salons A/B/C/D Room, 5th Floor:** Epidemiologists: we're not the same as statisticians.	**A. O'Connor**; Iowa State University, Ames, IA, USA.
8:45	**094**	Detection of *Salmonella enteritidis* in poultry environmental samples using a pooled real-time PCR assay.	**D. Adams**, W. Stensland, K. Harmon, E. Strait, C. Wang, T. Frana; Dept. of Veterinary Diagnostic and Production Animal Medicine, Iowa State University, Ames, IA, USA.
9:00	**095**	Genetic relatedness of *Salmonella* recovered from Michigan dairy farms in 2000 and 2009.	**G. Habing**, J.B. Kaneene; Center for Comparative Epidemiology, College of Veterinary Medicine, Michigan State University, East Lansing, MI, USA.
9:15	**096**	*Salmonella* in lymph nodes of cattle presented for harvest.	**S.E. Gragg**[1], G.H. Loneragan[1], M.M. Brashears[1], T.M. Arthur[2], J.M. Bosilevac[2], N. Kalchayanand[2], R. Wang[2], J.W. Schmidt[2], J.C. Brooks[1], S.D. Shackelford[2], T.L. Wheeler[2], T.R. Brown[1], D.M. Brichta-Harhay[2]; 1Texas Tech University, Lubbock, TX, USA, 2USDA, ARS, U.S. Meat Animal Research Center, Clay Center, NE, USA.
9:30		**Break and Table Top Exhibits – Foyer**	
		Presiders: Mohamed Fakr	
10:00 Tues.	**097**	Stress-adaptation can influence virulence in *Campylobacter jejuni*.	**G. Kumar-Phillips**[1], I. Hanning[2], M. Slavik[1]; 1Poultry Science, University of Arkansas, Fayetteville, AR, USA, 2Food Science & Technology, University of Tennessee, Knoxville, TN, USA.
10:15	**098**	The relationship between the occurrence of *Campylobacter* in post-chill carcasses and flock prevalence at various sampling points in broiler production and processing.	**K.L. Hataway**[1], J.A. Byrd[2], V.V. Volkova[3], S. Hubbard[1], D. Magee[1], R.H. Bailey[1], R.W. Wills[1]; 1Mississippi State University College of Veterinary Medicine, Mississippi State, MS, USA, 2SPARC, USDA ARS, College Station, TX, USA, 3Cornell University, Ithica, NY, USA.
10:30	**099**	Swine MRSA isolates form robust biofilms.	**T.L. Nicholson**; National Animal Disease Center-ARS-USDA, Ames, IA, USA.
10:45 Tues.	**100**	Longitudinal study of veterinary students for acquisition of methicillin-resistant *Staphylococcus aureus* associated with exposure to pork production facilities.	**A. Beahm**, J. Kinyon, L. Layman, L. Karriker, A. Ramirez, T. Frana; Dept. of Veterinary Diagnostic and Production Animal Medicine, Iowa State University, Ames, IA, USA.
11:45 to	**12:30**	**Business Meeting, Dedication, New Members Introduction, and Graduate Student Competition Awards Presentations**	

GASTROENTERIC DISEASES

Michigan/Michigan State Room - 6th Floor

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

Time	No.	Title	Authors
8:00 Mon.	101	Identification of PPK-1 and PPK-2 dependent transcriptome responses in *Campylobacter jejuni.*	**K. Chandrashekhar***[1], L. Heisler[2], D. Gangaiah[1], C. Nislow[3], S. Wijeratne[4], A. Wijeratne[4], T. Meulia[4], G. Rajashekara[1]; 1Food Animal Health Research Program-OARDC, The Ohio State University, Wooster, OH, USA, 2Donnelly Centre for Cellular and Biomolecular Research University of Toronto 160 College St, Toronto, ON, Canada, 3Donnelly Centre for Cellular and Biomolecular Research University of Toronto, Toronto, ON, Canada, 4Molecular and Cellular Imaging Center-OARDC, The Ohio State University, Wooster, OH, USA.
8:15	102	Loop mediated isothermal amplification method for detection of *Lawsonia intracellularis.*	**Y. Chander**, F. Vannucci, A. Rovira, C. Gebhart; University of Minnesota, Saint Paul, MN, USA.
8:30	103	*Lawsonia intracellularis* increases *Salmonella enterica* levels in the intestines of pigs.	**R. Isaacson**, K. Borewicz, H.B. Kim, F. Vannucci, C. Gebhart, R. Singer, S. Sreevatsan, T. Johnson; University of Minnesota, St. Paul, MN, USA.
8:45-9:30 Keynote	104	Mucosal immune system development in the small intestine of the newborn calf: regional differences in innate and acquired immunity.	**P.J. Griebel**[1], P.N. Fries[2]; 1Vaccine & Infectious Disease Organization, University of Saskatchewan, Saskatoon, SK, Canada, 2School of Public Health, University of Saskatchewan, Saskatoon, SK, Canada.
9:30		**Break and Table Top Exhibits – Foyer**	
10:00 Mon.	105	Transcriptional profiling of a pathogenic and an attenuated homologous Lawsonia intracellularis isolate during *in vitro* infection.	**F.A. Vannucci**, C.J. Gebhart; University of Minnesota, St. Paul, MN, USA.
10:15	106	A novel circular DNA virus from bovine stool is similar to chimpanzee stool-associated circular DNA virus, suggesting a new genus of circular DNA viruses.	**H.-K. Kim**, S.-J. Park, V.-G. Nguyen, H.-C. Chung, B.-K. Park; Seoul National University, Seoul, Korea, Republic of.
10:30	107	Quantitative evaluation of changes in C-reactive protein level and *Salmonella enterica* status as indicators of the swine health status in response to use of antibiotic growth promoter, Tylosin.	**H. Kim**[1], K. Borewicz[1], B.A. White[2], R.S. Singer[1], S. Sreevatsan[1], L.A. Espejo[3], R.E. Isaacson[1]; 1Dept. of Veterinary and Biomedical Sciences, College of Veterinary Medicine, University of Minnesota, Saint Paul, MN, USA, 2Dept. of Animal Sciences, University of Illinois, Urbana, IL, USA, 3Dept. of Veterinary Population Medicine, College of Veterinary Medicine, University of Minnesota, Saint Paul, MN, USA.
10:45 Mon.	108	Does infection caused by a multidrug resistant organism influence antimicrobial use practices in equine colic patients that had surgery?	**H. Aceto**, J.K. Linton, B.L. Dallap-Schaer; University of Pennsylvania School of Veterinary Medicine, Kennett Square, PA, USA.

IMMUNOLOGY

Salons F/G/H - 5th Floor

Section Leader: Isis Mullarky and Laura C. Miller

Time	No.	Title	Authors
		Presiders: Katherine Petersson and Susan Eicher	
8:00 Mon.	**109**	A specific CpG site demethylation in the IFN-gamma gene promoter region of different aged equine.	**Z. Gong**[1], L. Sun[2], D. Horohov[2]; 1Department of Biochemistry,Shanghai University of Traditional Chinese Medicine, Shanghai, China, 2Gluck Equine Research Center, Lexington, KY, USA.
8:15	**110**	The DNA promoter of the interferon gamma gene (Ifng) is hypermethylated in neonatal foals.	**L. Sun**[1], Z. Gong[2], D. Horohov[1]; 1Gluck Equine Research Center, Lexington, KY, USA, 2Department of Biochemistry,Shanghai University of Traditional Chinese Medicine, Shanghai, China.
8:30	**111**	The effect of flunixin meglumine on the equine immune response to vaccination.	**W.M. Zoll**[1], J. Dunham[2], A. Betancourt[2], A. Page[2], S. Reedy[2], T. Chambers[2], D. Horohov[2]; 1School of Veterinary Medicine, Michigan State University, Lawton, MI, USA, 2M.H. Gluck Equine Research Center, Lexington, KY, USA.
8:45	**112**	The maturation of equine infectious anemia virus (EIAV) envelope-specific immune responses in vivo after exposure to a live-attenuated vaccine.	**C. Liu**[1], S.J. Cook[1], J.K. Craigo[2], C.J. Issel[1], R.C. Montelaro[2], D.W. Horohov[1]; 1University of Kentucky, Lexington, KY, USA, 2University of Pittsburgh, Pittsburgh, PA, USA.
9:00	**113**	T regulatory cells and IgE are inversely correlated in horses vaccinated with viral vaccines.	**N.E. Behrens**, L.J. Gershwin; University California Davis, Davis, CA, USA.
9:15	**114**	Deacylated polyethyleneimine and IL-15 expression constructs modulate humoral and cellular immune responses to DNA vaccination in horses.	**D.L. Even**, C.J. Issel, S.J. Cook, R.F. Cook; University of Kentucky, Lexington, KY, USA.
9:30		**Break and Table Top Exhibits – Foyer**	
		Presiders: Carol Chitko-Mckown and Tracy Nicholson	
10:00 Mon.	**115**	Efficacy of attenuated Salmonella enterica serovar Typhimurium SA186, deleted of the zinc transporter ZnuABC, to control pig salmonellosis.	**P. Pasquali**[1], M. Pesciaroli[2], M. Gradassi[3], M.G. Zanoni[3], N. Martinelli[3], C. Pistoia[2], P. Petrucci[2], G. Lombardi[3], S. Ammendola[4], A. Battistoni[4], S. Thevasagayam[5], L.G. Alborali[6]; 1Istituto Superiore di Sanità, Rome, Italy, 2Istituto Superiore di Sanit, Rome, Italy, 3Istituto Zooprofilattico Sperimentale della Lombardia e dellEmilia Romagna, Brescia, Italy, 4Dipartimento di Biologia, Universit di Roma Tor Vergata, Rome, Italy, 5Pfizer Ltd. Animal Health, Paris, France, Paris, France, 6Istituto Zooprofilattico Sperimentale della Lombardia e dellEmilia Romagna, Rome, Italy.

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IMMUNOLOGY

Salons F/G/H - 5th Floor

Section Leader: Isis Mullarky and Laura C. Miller

Time	No.	Title	Authors
10:15 Mon.	116	Modulation of MHC I & II and cytokine expression by EHV-1 ORF 1/2 at the respiratory epithelium	**G. Soboll Hussey**[1], G. van de Walle[2], L.V. Ashton[1], A.M. Quintana[1], N. Osterrieder[3], D. Lunn[1]; 1Colorado State University, Department of Clinical Sciences, Fort Collins, CO, USA, 2Department of Comparative Physiology and Biometrics, Ghent University, Belgium, 3Freihe Universitaet Berlin, Berlin, Germany.
10:30	117	Differential regulation of mucosal immune responses at various mucosal tissues in pigs infected with *PRRSV* strain *VR2332.*	**C. Manickam**, V. Dwivedi, R. Patterson, K. Dodson, R. Gourapura; The Ohio State University, wooster, OH, USA.
10:45	118	Comparison of serological assays for *Actinobacillus pleuropneumoniae* (serotypes 1-9) on serum from pigs experimentally infected with APP or vaccinated with APP bacterins.	**M. Hemann**, S. Heinen, J. Johnson, P.G. Halbur, T. Opriessnig; Iowa State University, Ames, IA, USA.
11:00	119	Early vaccination of 5-day-old piglets does not alter the efficacy of two commercial porcine circovirus type 2 vaccines in an experimental triple infection challenge model.	**K.C. O'Neill, Jr.**[1], H. Shen[1], X. Lin[1], M. Hemann[1], N. Beach[2], X.-J. Meng[2], P. Halbur[1], T. Opriessnig[1]; 1Iowa State University, Ames, IA, USA, 2Virginia Polytechnic Institute, Blacksburg, VA, USA.
11:15	120	Nanoparticles entrapped killed *PRRSV* vaccine reduces *PRRSV* viremia in both homologous and heterologous *PRRSV* challenged pigs.	**V. Dwivedi**, C. Manickam, B. Binjawadagi, R. Patterson, R. Gourapura; The Ohio State University, Wooster, OH, USA.
11:30		**Lunch Break**	
		Presiders: Isis Mullarky and Lorraine Sordillo	
1:30-2:15 Mon. Keynote	121	**Distinguished Veterinary Immunologist:** Immunology and Animal Health: the whole is greater than the sum of its parts.	**Dr. Patricia E. Shewen**, Department of Vet. Pathobiology, University of Guelph, Guelph, Ontario, Canada.
2:15	122	The reduction of bacterial mastitis severity by treatment with 25-hydroxyvitamin D3.	**J.D. Lippolis**[1], T.A. Reinhardt[1], R.A. Sacco[1], B.J. Nonnecke[1], C.D. Nelson[2]; 1National Animal Disease Center / ARS / USDA, Ames, IA, USA, 2University of Wisconsin-Madison, Madison, WI, USA.
2:30	123	Mechanisms behind *Mycobacterium avium* subspecies paratuberculosis suppression of host cell apoptosis in primary bovine macrophages.	**P.M. Coussens**, E. Kabara; Michigan State University, East Lansing, MI, USA.
2:45		**Break and Table Top Exhibits – Foyer**	
		Presiders: John Lippolis and Lorraine Sordillo	
3:00	124	Bovine macrophages produce extracellular traps in response to Mannheimia haemolytica and its leukotoxin.	**N.A. Aulik**[1], K.M. Hellenbrand[2], C.J. Czuprynski[2]; 1Winona State Univeristy, Winona, MN, USA, 2Univeristy of Wisconsin-Madison, Madison, WI, USA.

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IMMUNOLOGY

Salons F/G/H - 5th Floor

Section Leader: Isis Mullarky and Laura C. Miller

Time	No.	Title	Authors
3:15 Mon.	**125**	Impaired capacity of neutrophils to produce reactive oxygen species, release extracellular traps and express genes encoding for cytokines may contribute to altered immune function in periparturient dairy cows.	**X. Revelo**, A. Kenny, N. Barkley, M. Waldron; University of Missouri, Columbia, MO, USA.
3:30	**126**	Selenoproteins alter eicosanoid biosynthesis in macrophages.	**S.A. Mattmiller**[1], B. Carlson[2], L. Sordillo[1]; 1Michigan State University, East Lansing, MI, USA, 2Molecular Biology of Selenium Section, National Cancer Institute, Bethesda, MA, USA.
3:45	**127**	Probing the effects of dual infections with *Mycobacterium avium* ss. *paratuberculosis* and bovine leukemia virus on Regulatory T cell prevalence and activity in cattle.	**J.A. Roussey**[1], B.N. Murphy[2], S.S. Sipkovsky[2], N. Turk[2], C.J. Colvin[2], P.M. Coussens[2]; 1Comparative Medicine and Integrative Biology Program, College of Veterinary Medicine, Michigan State University, East Lansing, MI, USA, 2Dept. of Animal Science, College of Agriculture and Natural Resources, Michigan State University, East Lansing, MI, USA.
4:00 Mon.	**128**	Proinflammatory responses of bovine endothelial cells to non-esterified fatty acids.	**W. Raphael**, G.A. Contreras, L.M. Sordillo; Michigan State University, East Lansing, MI, USA.
4:15	**129**	Inhibition of Mycoplasma mycoides subsp. mycoides adherence to transformed bovine skin fibroblasts by Mycoplasma specific monoclonal antibodies.	**R. Aye**[1], F. Chuma[1], M. Mwirigi[2], J. Naessens[1]; 1International Livestock Research Institute, Nairobi, Kenya, 2Kenya Agricultural Research Institute, Nairobi, Kenya.
4:30 to	**5:00**	**Break and Table Top Exhibits – Foyer**	
5:00 to	**6:30**	**Poster Session II Grand Ballroom Salon III - 7th floor**	

IMMUNOLOGY

Salons F/G/H - 5th Floor

Section Leader: Isis Mullarky and Laura C. Miller

Time	No.	Title	Authors
		Presiders: Laura Miller and Radhey Kaushik	
8:45 Tues.	**130**	Attenuation and protective efficacy of live attenuated *Salmonella* Gallinarum vaccines by employing a regulated delayed attenuation strategy.	**A. Mitra**, C. Willingham, A. Loh, A. Gonzales, R. Curtiss, III, K.L. Roland; The Biodesign Institute, Tempe, AZ, USA.
9:00	**131**	Intranasal vaccination with Ad5-encoding influenza HA elicits sterilizing immunity to homologous challenge and partial protection to heterologous challenge in pigs.	**D.R. Braucher**[1], J.N. Henningson[1], C.L. Loving[1], A.L. Vincent[1], E. Kim[2], J. Steitz[2], A.A. Gambotto[2], M.E. Kehrli, Jr.[1]; 1Virus and Prion Research Unit, National Animal Disease Center-USDA-ARS, Ames, IA, USA, 2Department of Surgery, University of Pittsburgh, Pittsburgh, PA, USA
9:15	**132**	Cytotoxic T cell responses in the spleen of infectious bursal disease virus infected chickens.	**A. Rauf**, M. Khatri, Y.M. Saif; The Ohio State Univesity, Wooster, OH, USA.
9:30		**Break and Table Top Exhibits – Foyer**	
		Presiders: Laura Miller and Radhey Kaushik	
10:00	**133**	Antiviral regulation underlying the activation status of porcine monocytic innate immune cells.	**Y. Sang**, R.R.R. Rowland, F. Blecha; Kansas State University, Manhattan, KS, USA.
10:15	**134**	Differentiation and immunoregulatory characteristics of porcine lung mesenchymal stem cells.	**M. Khatri**, Y.M. Saif; Ohio State University, Wooster, OH, USA.
10:30 Tues.	**135**	Sandwich -ELISA for diagnostics of African Swine Fever.	V.V. Tsibezov[1], Y.O. Terekhova[1], O.A. Verkhovsky[1], **A.D. Zaberezhny**[2], T.I. Aliper[3], E.A. Nepoklonov[1]; 1DPRI, Moscow, Russian Federation, 2D.I.Ivanovski Virology Institute, Moscow, Russian Federation, 3NARVAC R&D, Moscow, Russian Federation
10:45	**136**	Fc expressed on the surface of the PED virus enhanced immunogenecity.	**H. Jang**; Komipharm International Co. LTD., Gyeonggido, Korea, Republic of.
11:45 to	**12:30**	**Business Meeting, Dedication, New Members Introduction, and Graduate Student Competition Awards Presentations**	

RESPIRATORY DISEASES

Indiana/Iowa Room 6th Floor

Section Leaders: Amelia Woolums and Christopher Chase

Time	No.	Title	Authors
		Presiders: Amelia Woolums and Christopher Chase	
8:00 Mon.	**137**	Adaptationof a commercial PRRS serum antibody ELISA to oral fluid specimens.	**A. Kittawornrat**[1], J. Prickett[1], C. Wang[1], C. Olsen[1], C. Irwin[1], Y. Panyasing[1], A. Ballagi[2], A. Rice[2], J. Johnson[1], R. Main[1], C. Rademacher[3], M. Hoogland[3], J. Zimmerman[1]; 1Iowa State University, ames, IA, USA, 2IDEXX Laboratories, Inc., Westbrook, ME, USA, 3Murphy-Brown LLC, ames, IA, USA
8:15	**138**	Diagnostic performance of a commercial PRRS serum antibody ELISA adapted to oral fluid specimens.	**A. Kittawornrat**[1], J. Prickett[1], C. Wang[1], C. Olsen[1], C. Irwin[1], Y. Panyasing[1], A. Ballagi[2], A. Rice[2], J. Johnson[1], R. Main[1], C. Rademacher[3], M. Hoogland[3], J. Lowe[4], J. Zimmerman[1]; 1Iowa State University, ames, IA, USA, 2IDEXX Laboratories, Inc., Westbrook, ME, USA, 3Murphy-Brown LLC, ames, IA, USA, 4Carthge Veterinary Service, Ltd., Carthage, IL, USA.
8:30	**139**	Diagnostic performance of a commercial PRRS serum antibody ELISA adapted to oral fluid specimens: longitudinal response in experimentally-inoculated populations.	**A. Kittawornrat**[1], J. Prickett[1], C. Wang[1], C. Olsen[1], C. Irwin[1], Y. Panyasing[1], A. Ballagi[2], A. Rice[2], J. Johnson[1], R. Main[1], R. Rowland[3], J. Zimmerman[1]; 1Iowa State University, ames, IA, USA, 2IDEXX Laboratories, Inc., Westbrook, ME, USA, 3Kansas State University, Manhattan, KS, USA
8:45	**140**	Evaluation of herd exposure methods to produce PRRSv-negative pigs from infected breeding herds.	**D. Linhares**[1], J. Cano[2], M. Torremorell[1], R. Morrison[1]; 1University of Minnesota, Saint Paul, MN, USA, 2Boehringer Ingelheim Vetmedica Inc, St Joseph, MO, USA.
9:00	**141**	Evaluation of immune responses to porcine reproductive and respiratory syndrome virus in pigs at day two post-infection.	V. Dwivedi[1], C. Manickam[1], B. Binjawadagi[1], D. Linhares[2], M. Murtaugh[2], **G.J. Renukaradhya**[1]; 1The Ohio State University, Wooster, OH, USA, 2University of Minnesota, St. Paul, MN, USA.
9:15	**142**	Attenuation of a virulent North American PRRS virus isolate on CD163-expressing cell lines, and demonstration of efficacy against a heterologous challenge.	**J.G. Calvert**, M.L. Keith, L.P. Taylor, D.S. Pearce, D.E. Slade, S. Rai, S.W. Newport, R.G. Ankenbauer; Pfizer Animal Health, Kalamazoo, MI, USA.
9:30		**Break and Table Top Exhibits – Foyer**	
10:00 Mon.	**143**	Antiviral effect of various mutagens against PRRS Virus.	**A. Khatun**[1], E.-J. Choi[2], C.-H. Lee[2], K.-J. Yoon[3], W. Kim[1]; 1Chonbuk National University, Jeonju, Korea, Republic of, 2Animal Plant Fisheries Quarantine and Inspection Agency, Anyang, Korea, Republic of, 3Iowa State University, Ames, IA, USA.

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RESPIRATORY DISEASES

Indiana/Iowa Room 6th Floor

Section Leaders: Amelia Woolums and Christopher Chase

Time	No.	Title	Authors
10:15 Mon.	144	Lipid-anchored membrane association of the PRRS virus GP4 glycoprotein and co-localization with CD163 in lipid rafts.	**D. Yoo**[1], Y. Du[1], A. Pattnaik[2], C. Song[1]; 1University of Illinois at Urbana-Champaign, Urbana, IL, USA, 2Nebraska Center for Virology, University of Nebraska-Lincoln, Lincoln, NE, USA.
10:30	145	Adaptation of a commercial blocking ELISA to the detection of antibodies against influenza A virus nucleoprotein (NP) in porcine oral fluid specimens.	**Y. Panyasing**, C. Irwin, C. Wang, A. Kittawornrat, J. Prickett, K. Schwartz, J. Zimmerman; Iowa State University, Ames, IA, USA.
10:45	146	Emergence of novel reassortant H3N2 swine influenza viruses with the 2009 pandemic H1N1 genes in the United States.	J. Ma[1], Q. Liu[1], H. Liu[1], W. Qi[1], J. Anderson[1], S. Henry[2], R. Hesse[1], J. Richt[1], **W. Ma**[1]; 1kansas state university, Manhattan, KS, USA, 2Abilene Animal Hospital PA, Abilene, KS, USA.
11:00	147	Evaluation of cross-protection of FluSure XP® against a heterologous gamma cluster H1N1 swine influenza virus challenge.	**M.C. Lenz**[1], V.J. Rapp-Gabrielson[1], T. Hildebrand[1], L. Taylor[1], M. Kuhn[2], M.R. Gramer[3]; 1Pfizer Animal Health, Kalamazoo, MI, USA, 2Pfizer Animal Health, Cascade, IA, USA, 3University of Minnesota Veterinary Diagnostic Laboratory, St. Paul, MN, USA.
11:15	148	Modified live virus vaccine induces a distinct immune response profile compared to inactivated influenza A virus vaccines in swine.	**P. Gauger**[1], A. Vincent[1], C. Loving[1], A. Lorusso[1], K. Lager[1], L. Pena[2], D. Perez[2]; 1National Animal Disease Center, Ames, IA, USA, 2University of Maryland, College Park, MD, USA.
11:30		**Lunch Break**	
1:30 Mon.	149	Evaluation of a commercial blocking ELISA kit for detection of influenza A nucleoprotein antibodies in canine sera.	**T.C. Anderson**, S.A. Salomon, P.C. Crawford; Department of Small Animal Clinical Sciences, College of Veterinary Medicine, University of Florida, Gainesville, FL, USA.
1:45	150	Pneumonia cases associated with *Mycoplasma hyopneumoniae*: a retrospective evaluation of diagnostic cases from 2003 to 2010.	**J.C. Gomes Neto**, N. Boyes, E.I. Strait, K.J. Schwartz, A. Ramirez; Veterinary Diagnostic and Production Animal Medicine, Iowa State University, AMES, IA, USA.
2:00	151	Increased prevalence of torque teno viruses in porcine respiratory disease complex affected pigs.	L. Ramohan[1], K. Lin[1], C. Wong[1], W. Chittick[1], S. Ganesan[1], **S. Ramamoorthy**[2]; 1Iowa State University, Ames, IA, USA, 2University of Georgia, Tifton, GA, USA.
2:15	152	The development and validation of two non-invasive diagnostic screening assays for the detection of tuberculosis infection in non-human primates.	**T.M. Wolf**, R. Singer, S. Sreevatsan; University of Minnesota, St. Paul, MN, USA.
2:30	153	Comparison of peptide cocktails and purified protein derivatives for use in the Bovigam™ assay.	**K.E. Bass**[1], B.J. Nonnecke[1], M.V. Palmer[1], T.C. Thacker[1], R. Hardegger[2], B. Schroeder[2], A.J. Raeber[2], W.R. Waters[1]; 1National Animal Disease Center, Ames, IA, USA, 2Prionics AG, Schlieren, Switzerland.

RESPIRATORY DISEASES

Indiana/Iowa Room 6th Floor

Section Leaders: Amelia Woolums and Christopher Chase

Time	No.	Title	Authors
2:45 Mon.		**Break and Table Top Exhibits – Foyer**	
3:00	**154**	Genome-wide analysis of gene expression profile change in response to BRSV and *H. somni* in bovine respiratory epithelial cells.	**M.X. Shao**[1], L.B. Corbeil[2], L.J. Gershwin[1]; 1University of California, Davis, CA, USA, 2University of California, San Diego, CA, USA.
3:15	**155**	BRSV and *H. somni* synergy in bridging the alveolar barrier.	**J. Agnes**[1], B. Zekarias[1], L.J. Gershwin[2], L.B. Corbeil[3]; 1UCSD, San Diego, CA, USA, 2UC Davis, Davis, CA, USA, 3UCSD and UC Davis, San Diego and Davis, CA, USA.
3:30 Mon.	**156**	Cytokine and chemokine responses of equine pulmonary alveolar macrophages are altered in a dose-dependent manner to *Rhodococcus equi* infection.	S. Hashimoto-Hill[1], M. Heller[2], K. Jackson[2], **J. Watson**[2]; 1Veterinary Teaching Hospital, University of California, Davis, CA, USA, 22Dept. of Medicine and Epdemiology, University of California, Davis, CA, USA.
3:45-4:30 Keynote	**157**	Infections caused by *Rhodococcus equi* in foals: immunologic and therapeutic considerations.	**S. Giguere**; University of Georgia, Athens, GA, USA.
4:30 to	**5:00**	**Break and Table Top Exhibits – Foyer**	
5:00 to	**6:30**	**Poster Session II Grand Ballroom Salon III - 7th floor**	

VECTOR-BORNE AND PARASITIC DISEASES

Denver/Houston - 5th Floor

Section Leader: Roman Ganta

Time	No.	Title	Authors
8:00 Mon.	158	Development of a sheep model for studying pathogen vector interactions of *Anaplasma phagocytophilum* and *Ixodes scapularis*.	**K.M. Kocan**, A.T. Busby, R.W. Allison, M.A. Breshears, E.F. Blouin, J. de la Fuente; Oklahoma State University, Stillwater, OK, USA.
8:15	159	Potentially protective dual oxidase enzymes (Duox1 and Duox2) in T.foetus infected bovine endometrial cells *in vitro*.	**B. Adu-Addai**[1], C. Mackenzie[1], A. Langerveld[2], D. Agnew[1]; 1Michigan State University, Lansing, MI, USA, 2Genemarkers, LLC, Kalamazoo, MI, USA.
8:30	160	Understanding the basis of strain-restricted immunity to Theileria parva.	**T. Sitt**[1], R. Pelle[1], L. Steinaa[1], I. Morrison[2], P. Toye[1]; 1International Livestock Research Institute, Nairobi, Kenya, 2University of Edinburgh, Edinburgh, UK.
8:45	161	Prevalence of tick-borne anaplasmal pathogens among naturally infected client-owned dogs in Missouri.	**S. YOO-EAM**[1], R. Stoffel[1], K. Curtis[1], M. Whitney[1], A. Bermudez[1], W. Roland[1], P. Rajala-Schulz[2], R. Stich[1]; 1University of Missouri, Columbia, MO, USA, 2The Ohio State University, Columbus, OH, USA.
9:00	162	Epidemiology of epizootic hemorrhagic disease in white-tailed deer in Texas.	**B. Szonyi**[1], A. Clavijo[2], R. Ivanek[1]; 1Texas A&M University, College Station, TX, USA, 2Texas Veterinary Medical Diagnostic Laboratory, College Station, TX, USA.
9:15	163	Functional analysis of tick genes differentially expressed in response to Anaplasma phagocytophilum infection.	**A.T. Busby**[1], N. Ayllón[2], M. Villar[2], K.M. Kocan[1], E.F. Blouin[1], R.C. Galindo[2], E. Bonzón-Kulichenko[3], J. Vázquez[3], J. José de la Fuente[1]; 1Oklahoma State University, Stillwater, OK, USA, 2Instituto de Investigación en Recursos Cinegéticos IREC (CSIC-UCLM-JCCM), Ciudad Real, Spain, 3Centro de Biología Molecular "Severo Ochoa" (CSIC-UAM), Madrid, Spain.
9:30		**Break and Table Top Exhibits – Foyer**	
10:00-10:45 Mon. Keynote	164	Plasmepsins: Dissecting the function of a parasite's protease degradome.	**J.B. Dame**; Infectious Diseases and Pathology, University of Florida, Gainesville, FL, USA.
10:45	165	Improvement in diagnostic specificity of *anaplasma marginale* msp5 epitope-based celisa with new antigen construct.	**C. Chung**; VMRD Inc., Pullman, WA, USA.
11:00	166	A novel *Theileria equi* sporozoite challenge model for pathogenesis and immune control studies in immunocompetent and immunodeficient horses.	**J.D. Ramsay**[1], M.W. Ueti[2], G.A. Scoles[2], D.P. Knowles[2], R.H. Mealey[1]; 1Dept. Veterinary Microbiology and Pathology, College of Veterinary Medicine, Washington State University, Pullman, WA, USA, 2Animal Disease Research Unit, USDA-ARS, Washington State University, Pullman, WA, USA.

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VECTOR-BORNE AND PARASITIC DISEASES

Denver/Houston - 5th Floor

Section Leader: Roman Ganta

Time	No.	Title	Authors
11:15 Mon.	167	Light microscopic study of the developmental cycle of *Ixodes scapularis.*	**K.M. Kocan**[1], L. Coburn[2], A.T. Busby[2], E.F. Blouin[2], J. de la Fuente[2]; 1Oklahoma State University, Stillwater,, OK, USA, 2Oklahoma State University, Stillwater, OK, USA.
11:30	168	Society for Tropical Veterinary Medicine (STVM) Presentation	**E.F. Blouin**; Veterinary Pathobiology, Oklahoma State University, Stillwater, OK, USA.
11:45		**Lunch Break**	
4:30 to	**5:00**	**Break and Table Top Exhibits – Foyer**	
5:00 to	**6:30**	**Poster Session II Grand Ballroom Salon III - 7th floor**	

VIRAL PATHOGENESIS

Los Angeles/Miami/Scottsdale Rooms - 5th Floor

Section Leader: Kyoung-Jin Yoon

Time	No.	Title	Authors
8:00-8:45 Mon. Keynote	169	Keynote Presentation: DIVA Vaccination for Avian Influenza Virus: Ready for Prime Time?	**David L. Suarez**; Southeast Poultry Research Laboratory, Athens, GA, USA.
8:45	170	Pathogenicity and cytokine gene expression patterns associated with fowl adenovirus serotype 4 infection.	**H. Grgic**, Z. Poljak, S. Sharif, É. Nagy; Ontario Veterinary College, Guelph, ON, Canada.
9:00	171	Genetic characterization of Newcastle disease viruses, allocated in Ukraine in 2006-2009.	**A.P. Gerilovych**, A.B. Stegniy, B.T. Stegniy; NSC Institute of experimental and clinical veterinary medicine, Kharkiv, Ukraine.
9:15	172	Misfolded Y145stop catalyzes the conversion of full prion protein.	**A.M. Abdallah**[1], P. Wang[1], J. Richt[2], S. Sreevatsan[1]; 1Department of Veterinary Population Medicine, University of Minnesota, Saint Paul, MN, USA, 2College of Veterinary Medicine, Kansas State University, Manhattan, KS, USA.
9:30		**Break and Table Top Exhibits – Foyer**	
10:00 Mon.	173	Pathogenicity and immune response in the lung of pigs experimentally infected with diverse genotype I PRRSV strains, including a pathogenic subtype 3 strain.	**S.B. Morgan**[1], S.P. Graham[1], P.J. Sanchez-Cordon[2], F. Steinbach[1], J.-P. Frossard[1]; 1Animal Health and Veterinary Laboratories Agency, Weybridge, UK, 2University of Cordoba, Cordoba, Spain.
10:15	174	Sequence and virulence comparison of four North American isolates of porcine reproductive and respiratory syndrome virus.	**S.L. Brockmeier**[1], C.L. Loving[1], L.C. Miller[1], A.C. Vorwald[1], M.E. Kehrli, Jr[1], R.B. Baker[2], T.L. Nicholson[1], K.M. Lager[1], K.S. Faaberg[1]; 1National Animal Disease Center, Ames, IA, USA, 2Iowa State University, Ames, IA, USA.
10:30	175	Isotype profile of PRRSV nucleocapsid-specific antibody response in pigs after experimental infection.	**S.G. Nezami**[1], D. Sun[1], A. Kittawornrat[1], R. Molina[2], S. Cha[3], R.R. Rowland[4], J.J. Zimmerman[1], K.-J. Yoon[1]; 1Iowa State University, Ames, IA, USA, 2Instituto Tecnologico de Sonora (ITSON), Ciudad Obregn, Mexico, 3National Veterinary Research and Quarantine Service, Anyang, Korea, Republic of, 4Kansas State University,National Veterinary Research and Quarantine Service, Manhattan, KS, USA.
10:45	176	Novel PRRSV ORF5a protein is not immunoprotective but drives GP5 glycosylation.	**S.R. Robinson**, M.C. Figueiredo, J.E. Abrahante, M.P. Murtaugh; University of Minnesota, St. Paul, MN, USA.

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VIRAL PATHOGENESIS

Los Angeles/Miami/Scottsdale Rooms - 5th Floor

Section Leader: Kyoung-Jin Yoon

Time	No.	Title	Authors
11:00 Mon.	177	Development of virus-like particle vaccine of porcine reproductive and respiratory syndrome virus and analysis of immune responses in the vaccinated mice.	**I.-S. Choi**[1], H.-M. Nam[1], Y.-J. Song[1], J.-B. Lee[1], S.-Y. Park[1], C.-S. Song[1], S.-M. Kang[2], M.-C. Kim[3]; 1Konkuk University, College of Veterinary Medicine, Seoul, Korea, Republic of, 2Georgia State University, Center for Inflammation, immunity and Infection Department of Biology, Atlanta, GA, USA, 3Emory University School of Medicine, Department of Microbiology and Immunology, Atlanta, GA, USA
11:15	178	Intranasal delivery of an adjuvanted modified live porcine reproductive and respiratory syndrome virus vaccine reduces the ROS induced lung pathology.	**B. Binjawadagi**[1], V. Dwivedi[1], C. Manickam[1], J.B. Torrelles[2], G.J. Renukaradhya[1]; 1Food Animal Health Research Program, Ohio Agricultural Research and Development Center, Department of Veterinary Preventive Medicine, The Ohio State University, Wooster, OH, USA, 2Center for Microbial Interface Biology, Division of Infectious Diseases, Department of Internal Medicine, The Ohio State University, Columbus, OH, USA.
11:30		**Lunch Break**	
1:30 Mon.	179	Effect of deoxynivalenol (DON) mycotoxin on porcine reproductive respiratory syndrome virus (PRRSV) *in vitro*.	**C. Savard**, V. Pinilla, C. Provost, M. Segura, C.A. Gagnon, Y. Chorfi; Universite de montreal, Saint-Hyacinthe, QC, Canada.
1:45	180	FISHing for cats: development of a fluorescence *in situ* hybridization (FISH) assay targeting feline papillomaviruses.	**L. Demos**[1], M. Bennett[1], J. Munday[2]; 1Murdoch University, Murdoch, Australia, 2Massey University, Palmerston North, New Zealand.
2:00	181	Sequence analysis of swine influenza viruses circulating in US before and after the pandemic 2009 H1N1 influenza outbreak.	S.G. Nezami, **D. Sun**, L.P. Bower, J. Zhang, D. Haney, K.-J. Yoon; Iowa State University, Ames, IA, USA.
2:15	182	Identification of novel swine/pandemic H1N1 reassortant virus in pigs.	**A. Ali**, M. Khatri, L. Wang, Y.M. Saif, C. Lee; The Ohio State University, Wooster, OH, USA.
2:30	183	*In vitro* reassortment between endemic H1N2 and pandemic 2009 H1N1 Swine Influenza Viruses	**B. Hause**[1], R. Simonson[1], F. Li[2]; 1Newport Labs, Worthington, MN, USA, 2South Dakota State University, Brookings, SD, USA.
2:45		**Break and Table Top Exhibits – Foyer**	
3:00 Mon.	184	Evaluation of cd25, foxp3, and ccl5 gene expression in placentomes of pregnant cattle inoculated with bovine viral diarrhea virus.	**H.L. Walz**, R.A. Palomares, J.M. Caldwell, P.H. Walz, K.V. Brock; Dept. of Pathobiology, College of Veterinary Medicine, Auburn University, Auburn, AL, USA.
3:15	185	PCV2 infection from birth through finishing.	**C.M.T. Dvorak**, M.P. Lilla, S.R. Baker, M.P. Murtaugh; University of Minnesota, St.Paul, MN, USA.

POSTER ABSTRACTS

BACTERIAL PATHOGENESIS POSTERS

001P

Correlation of foot conformation with likelihood of development of digital dermatitis in lactating dairy cattle.

C. Haglund, A. Krull, J. Rabenold, P. Gorden, B. Leuschen, J. Shearer, P. Plummer;
Iowa State University, Ames, IA, USA.

Digital dermatitis (DD) is a bacterial disease process primarily associated with the hind feet of cattle. Since first documented in 1974, DD has become a leading cause of lameness in dairy cattle, resulting in major economic and animal welfare concerns. Our lab is currently investigating the possibility of a polymicrobial interaction as the disease's cause. This project's first objective is to determine if the physical conformation of the cow foot is associated with an increased risk of DD lesion development. Our hypothesis is that cows with shorter heels or redundant skin folds in the interdigital space will harbor larger bacterial loads of suspected pathogens due to their heel's closer proximity to fecal slurry (a potential source of bacterial pathogens) and the anaerobic environment established in the tissue folds. To test this hypothesis, we measured the heel height, angle of the ankle, and presence/depth of a pocket in the interdigital space. The results suggest that pocket depth has a significant correlation with
lesion development, but heel height and ankle angle do not. As a second objective, we investigated two non-invasive techniques to sample bacterial populations in DD lesions. Based on the superficial nature of many of the DD bacterial organisms, we hypothesized that washes or swabs of lesions would correlate well with biopsy PCR results. We collected samples, using the two noninvasive techniques, as well as biopsies on multiple lesions and compared the three using PCR. We found that these non-invasive collection techniques were comparable to biopsies in collection of bacterial samples in stage 3-4 lesions, but can also identify some potential bacterial pathogens in stage 1-2 lesions that biopsies do not.

002P

The potential symbiotic relationship of anaerobic bacteria along with *Treponema* spp. in the development of papillomatous digital dermatitis.

A. Krull, J. Rabenold, M. Elliot, P. Gorden, J. Shearer, B. Leuschen, P. Plummer;
Iowa State University, Ames, IA, USA.

Purpose: Papillomatous Digital Dermatitis (PDD) is recognized as a significant cause of lameness in dairy cattle throughout the US and industrialized world. Published research has demonstrated that several treponemal species (ie. pedis, medium ssp. bovis, phagedenis) are routinely isolated from these lesions, however intervention strategies targeting these bacterial species have failed to provide consistent protection. Given the complexity of the bacterial populations found in bovine fecal slurry to which these lesions are continually exposed, we hypothesize that these lesions are likely associated with a mixed bacterial infectious process. Methods: In order to test this hypothesis, we have initiated studies focused on recognizing the developmental stages of PDD lesions and identifying intra-lesional bacteria utilizing a variety of culture dependent and culture independent methods. The culture dependent methodology relies on utilization of eight anaerobic media (both selective and non-selective) inoculated with biopsies obtained from active or developing PDD lesions. Results: In addition to the expected treponemal species, we consistently culture several bacterial species from PDD lesions using anaerobic media. Based on 16S sequencing we have identified several bacterial genera including *Porphyromonas* spp. that have been previously demonstrated to grow synergistically with treponemes in human gingivitis. These same organisms have also been cultured from lesions in early developmental stages prior to identification as a classic PDD lesion. Conclusions: The consistent presence of *Porphyromonas* and *Bacteroides* spp. in addition to treponemes in PDD lesions suggest that these organisms may be important in this disease process. Additional studies are currently underway to determine the clinical significance of these organisms in PDD.

003P

Identification and characterization of genes required for *Campylobacter* resistance to Fowlicidin-1, a chicken host defense peptide.

K.V. Hoang, J. Lin;
University of Tennessee, Knoxville, TN, USA.

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 largely unknown in *C. jejuni*, an important human foodborne pathogen with poultry as a major reservoir. 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. In addition, chicken experiment was performed to determine the role of candidate genes in *Campylobacter* colonization in the intestine. An efficient transposon mutagenesis approach in conjunction with a microtitre 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 two-component regulator CbrR, transporter CjaB, and a putative trigger factor (Tig). 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 also significantly increased susceptibility of *Campylobacter* to fowlicidin-1 in diverse strain background. Chicken experiment showed that these mutants displayed reduction or defect in *in vivo* colonization. Together, these results have defined 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.

004P

Enzymatic characteristics of a novel enterobactin esterase Cee in Campylobacter.

Y. Mo, X. Zeng, J. Lin;
Department of Animal Science, University of Tennessee, Knoxville, TN, USA.

We recently identified a novel periplasmic enterobactin (Ent) esterase Cee that plays a critical role in Ent-mediated iron acquisition in *Campylobacter*. However, catalytic efficiency and kinetics of Cee are still unknown. In this study, we purified Cee and its homologs (IroE and IroD from *E. coli*) for enzymatic analysis using thin layer chromatography (TLC) and high performance liquid chromatography (HPLC). Both TLC and HPLC analyses showed that Cee displayed exceptionally high efficiency to hydrolyze Ent. Unlike the periplasmic IroE that tends to hydrolyze Ent just once to produce linearized trimers, Cee could efficiently catalyze the hydrolysis of Ent, generating linear trimer, dimmer, and monomer products. Notably, dimer and monomer products were generated as early as 1 min after Cee treatment; hydrolysis of Ent with Cee for 10 min led to dimers and monomers with no linear trimer and cyclic Ent left. The catalytic efficiency of Cee also seemed to be higher than the well characterized cytoplasmic IroD and Fes in *E. coli*. Together, this study reveals unique features of Cee when compared to its homologs in other bacteria, and strongly supports an uncharacterized and fascinating process for Ent-mediated iron acquisition in Gram-negative bacteria.

BACTERIAL PATHOGENESIS POSTERS

005P
Characterization of enterobactin esterase Cee in *Campylobacter* led to a novel model of ferric enterobactin iron acquisition.
X. Zeng, F. Xu, Y. Mo, J. Lin;
University of Tennessee, Knoxville, TN, USA.

Ferric enterobactin (FeEnt) acquisition system plays a critical role in *Campylobacter* pathogenesis. Interestingly, although *C. jejuni* JL11 and 81-176 share similar machinery for FeEnt uptake, only JL11 efficiently utilizes FeEnt as a sole iron source for growth. Thus, we hypothesize that JL11 contains an unidentified component that is essential for FeEnt acquisition with this component missing in 81-176. In this study, the JL11 genome was sequenced by 454 GS FLX sequencer. Gaps were closed between contigs and the draft genome sequence was subjected to automatic annotation. Comparative genomic analysis of JL11, 81-176 and NCTC 11168 genomes suggested that Cj1376 is the missing component in 81-176 required for FeEnt assimilation. Complementation of 81-176 with *cj1376* completely restored its ability to utilize FeEnt. Sequence analysis showed that Cj1376 is a periplasmic protein displaying low but significant homology to the Ent esterases identified in other bacteria, such as IroE in *Salmonella*; therefore, Cj1376 was designated Cee (*Campylobacter* enterobactin esterase). The crude extract from 11168 effectively catalyzed the hydrolysis of Ent but the extract from isogenic *cee* mutant failed to hydrolyze Ent. Thin layer chromatography demonstrated that the periplasmic Cee displayed exceptionally high efficiency to hydrolyze Ent, which is distinct from its homolog IroE. Amino acid substitution mutagenesis revealed that Ser157 and His251 were essential residues for Cee. Genetic analysis and manipulation of various *Campylobacter* strains demonstrated that Cee is not only essential for CfrB-dependent FeEnt acquisition but also involved in CfrA-dependent pathway in *Campylobacter*. Inactivation of *cee* dramatically reduced and even abolished colonization of *Campylobacter* in the intestine. Together, this research identified a novel periplasmic Ent esterase Cee, revealed unique features of *Campylobacter* FeEnt acquisition, and suggested a new model that is different from the widely conserved process of FeEnt acquisition in other bacteria.

006P
Complex molecular interaction of ferric enterobactin acquisition in *campylobacter*: role of TonB-ExbB-ExbB energy transduction system.
X. Zeng, F. Xu, J. Lin;
University of Tennessee, Knoxville, TN, USA.

We recently identified and characterized two ferric enterobactin (FeEnt) acquisition systems that play a critical role in *Campylobacter* iron utilization and *in vivo* colonization. Specific outer membrane receptor, CfrA or CfrB, is required for initial high-affinity binding of FeEnt. Following binding, FeEnt must be transported through the receptor pore using TonB-ExbB-ExbD energy transduction system. In *Campylobacter*, there are up to three TonB-ExbB-ExbD systems. However, specific interaction between the FeEnt receptors and the TonB systems is still largely unknown. In this study, the cognate TonB-ExbB-ExbD systems of CfrA- and CfrB-dependent FeEnt acquisition were determined using genomic analysis, site-directed mutagenesis, complementation, and random transposon mutagenesis. Complementation of *C. jejuni* 81-176 (only containing TonB2-ExbB2-ExbD2) with *cfrA-tonB3* operon, but not *cfrA* alone, completely rescued its ability to utilize FeEnt. Inactivation of *tonB3*, *exbB2*, or *exbD2* alone in NCTC 11168 completely abolished its ability to utilize FeEnt via CfrA-dependent pathway, further indicating that CfrA specifically interacts with TonB3-ExbB2-ExbD2 system in *Campylobacter*. With respect to CfrB-dependent pathway, ExbB2 and ExbD2 were essential but CfrB could interact with either TonB2 or TonB3 for efficient iron uptake. Our findings also suggest that other components, including TonB1, ExbB1-ExbD1, and ExbB3-ExbD3, are not involved in FeEnt acquisition in *Campylobacter*. In conclusion, these findings reveal identities of specific TonB-ExbB-ExbD energy transduction systems required for FeEnt acquisition, and provide insights into the complex molecular interactions of FeEnt acquisition systems in *Campylobacter*.

007P
Does *Campylobacter* use intracellular reductive iron release mechanism to utilize ferric enterobactin?
X. Zeng, J. Lin;
University of Tennessee, Knoxville, TN, USA.

Siderophore-mediated iron scavenging is critical for bacterial pathogenesis. Release of iron from siderophore is the last and essential step in siderophore-mediated iron scavenging. To utilize ferric enterobactin (FeEnt), the complex with extremely low redox potential, hydrolytic iron release mediated by cytoplasmic esterase (e.g. Fes in *E. coli*) is believed to be a common and powerful mechanism. Recently, we have identified a periplasmic Ent esterase Cee in *Campylobacter*. However, the absence of a Fes-like intracellular Ent esterase in *Campylobacter* genome suggests that this organism may use ferric reductase to release iron from Ent, a mechanism for releasing iron from the siderophores with high redox potential. The published microarray data indicated that *cj1377c*, a gene immediately downstream of *cee*, is induced by iron limitation, suggesting Cj1377c is involved in iron metabolism. Annotation analysis showed Cj1377c is a Fe-S protein containing a highly conserved domain (TIGR03224, E-value of 2e-07) represented by BoxA, a ferredoxin-NADPH reductase. Since ferredoxin-NADPH reductase can function as a ferric reductase, Cj1377c is likely a ferric reductase required for FeEnt acquisition in *Campylobacter*. To test this, a non-polar isogenic *cj1377c* mutant of NCTC11168 was generated and evaluated for its ability to utilize FeEnt. The Ent growth promotion assay demonstrated that inactivation of *cj1377c* almost completely abolished the ability of NCTC11168 to utilize FeEnt. The *fldA* (*cj1382c*), an essential gene adjacent to *cj1377c*, may also involve reductive iron release from FeEnt due to the presence of a highly conserved FMN_red super family domain in FldA. Together, this study demonstrated that Cj1377c, a putative ferric reductase, is involved in FeEnt acquisition in *C. jejuni*. High-purity Cj1377c and FldA need to be produced for examination of their specific role in intracellular reductive iron release from FeEnt, a process that has never been clearly demonstrated in other bacteria.

008P
Mutation of luxS gene in *Campylobacter jejuni* impacts major virulence attributes.
K. Mou, P. Plummer;
Iowa State University, Ames, IA, USA.

Purpose: The AI-2/LuxS system has been associated with expression of key virulence factors in many bacterial pathogens. Campylobacter species possess such a system and previous studies have found the importance of LuxS system for colonization and/or translocation of the organism through the intestinal barrier to enter systemic circulation. This study used a mechanistic approach to understand how LuxS system is involved in the expression of virulence factors key to C. jejuni colonization and invasion of its host.
Methods and results: Images of a previously constructed luxS mutant of C. jejuni NCTC 11168 were compared with the wild-type using Imagestream® imaging cytometer. The mutant strain exhibited a significantly smaller cell length than the wild-type. The smaller size may compromise its helical cell shape that is necessary for successful colonization of the thick gastric mucosa. Another factor important for C. jejuni adaptation in the host, efflux pumps, was also found to be affected by the luxS mutation. Real-time data from a growth study (under basal conditions) of luxS and three different efflux pump mutants showed most had at least two-fold changes in luxS expression levels. Although minimal changes in expression levels for one of the major efflux pump genes, cmeB, were observed in the luxS mutant, the mutant demonstrated higher efflux activity than its wild-type and luxS complement. This could

008P (continued)
indicate involvement of efflux systems aside from CmeABC system that would be responsible for the high efflux activity of luxS mutant. Such alterations in luxS expression levels and efflux activity may indicate an attempt to maintain normal cellular homeostasis and metabolic activity.
Conclusions: The luxS mutation showed appreciable effects on efflux pump systems and cell size, important virulence factors necessary for successful colonization of the host. These results may provide an explanation for LuxS mutant's inability to colonize its host as evidenced in recent publications.

009P
Identification of Iron Acquisition Machinery of Flavobacterium columnare.
J. Santander, L. Guan, M. Mellata, R.I. Curtiss;
Arizona State University, Tempe, AZ, USA.

Purpose: Flavobacterium columnare, a fastidious Gram-negative pathogen, is the causative agent of the columnaris disease, a globally distributed infection of freshwater and marine water fish. Despite efforts made on understanding the mechanism of virulence of F. columare, much remains poorly understood. The ability of bacteria to take up iron from the host during the infection process is necessary for their multiplication within the host. Herein we identified and analyzed the iron uptake machinery of F. columnare. Methods and Results: In absence of iron in in vitro growth, an outer membrane protein of ~96 kDa was upregulated in F. columnare. This protein was identified as a ferrichrome-iron receptor (FhuA) TonB dependent protein, and confirmed as a siderophore receptor using structural analysis. Synthesize of siderophores in this bacteria in absence of iron was corroborated by chrome azurol assays. F. columnare genome presents a putative ferric uptake regulator (fur) gene with similar protein structure than other Fur proteins involved in iron uptake regulation in other bacteria. Salmonella enterica Δfur mutants were partially complemented by F. columnare fur gene. Conclusions: We conclude that F. columnare presents a siderophore iron uptake system most likely regulated in a Fur dependent fashion.

010P
Identification of *Streptococcus uberis* mutants susceptible to neutrophil killing.
J. Warren, O. Kerro Dego, R. Almeida, S.P. Oliver, G.M. Pighetti;
University of Tennessee, Knoxville, TN, USA.

The most detrimental disease affecting the dairy industry worldwide is mastitis. This disease results in substantial losses in profit every year to dairy producers. While there are many causes of this disease, one leading cause is infection with *Streptococcus uberis*. One reason for the difficulty in controlling this pathogen is due to its resilience against neutrophil killing. Purpose: The objectives of this study are to 1) identify clones of *S. uberis* UT888 which contain mutations that allow neutrophils to destroy the pathogen and 2) identify the mutated genes in one or more of the top candidates. Methods: A transposon-generated mutant library of *S. uberis* containing 1,000 clones will be screened for mutants susceptible to neutrophil killing. As a preliminary screen, *S. uberis* was incubated in the presence or absence of freshly isolated bovine neutrophils from Holstein heifers. Viable *S. uberis* was measured via reduction of MTT and compared against a standard curve generated from the wild type strain UT888. Results: The preliminary screening of 192 clones found 2 mutated clones that were killed more effectively than the wild type UT888 (25% and 106%). Each mutant clone was greater than 2.5 SD beyond the mean killing of all clones located on the same 96 well plate. Conclusion: Based on this, we predict approximately 10 mutants susceptible to neutrophil killing will be found in a 1000 clone library. Identifying the genes and processes that *S. uberis* uses to resist killing by neutrophils will provide critical knowledge to controlling this infection.

011P
Expression patterns of virulence genes of *E. coli* strains associated with acute and chronic bovineintramammary infections.
O. Kerro Dego, S.P. Oliver, R.A. Almeida;
The University of Tennessee, Knoxville, TN, USA.

Escherichia coli intramammary infections (IMI) show acute local and systemic clinical symptoms that often clear within 7 days. However, if not detected and treated early, *E. coli* IMI results in generalized systemic conditions that could lead to death. Chronic *E. coli* IMI is characterized by mild clinical manifestations followed by episodes of clinical mastitis during lactation. Factors responsible for pathogenesis of *E. coli* IMI and variation in clinical manifestations are not known. There were studies indicating that the outcome of *E. coli* IMI is mainly determined by cow factors. However, recent studies demonstrated that virulence attributes of *E. coli* strains have a significant impact on the outcome of *E. coli* IMI. We hypothesize that the pathogenesis and clinical severity of *E. coli* IMI is determined by combined effects of host-pathogen factors. The aim of this study was to identify genes of *E. coli* induced during bacterial interaction with primary mammary epithelial cells (PBMEC). We evaluated expression patterns of virulence associated genes of *E. coli* after co-culture with PBMEC using qRT-PCR. Our results showed that interaction of acute or chronic strains with PBMEC induced significant up-regulation of aerobactin siderophore ferric receptor (*iutA*), intimin (*eae*), outer membrane porin protein precursor (*onmPC*), outer membrane protein II (*ompA*), succinate dehydrogenase (*sdh*) and LEE-encoded regulator (*ler*). Known virulence genes such as *eae*, *iutA* and *ler* showed higher expression in the chronic than in the acute strain. Results suggest that the clinical severity of *E. coli* IMI is influenced by the degree of expression of pathogen factors.

012P
Immunoproteome analysis of the outer membrane proteins of *Salmonella* Gallinarum.
J. Sun[1], J. Han[2], J. Jang[3], E. Hong[1], T. Hahn[1];
[1]Kangwon National University, College of Veterinary Medicine, Chuncheon, Korea, Republic of, [2]KBNP Technology Institute, KBNP Inc, an, Korea, Republic of, [3]KBNP Technology Institute, KBNP Inc., Yeasn, Korea, Republic of.

Purpose: Fowl Typhoid (FT) is a septicemic diseases caused by *Salmonella* Gallinarum(SG). The objective of this study was to screen the OMPs of SG to identify the proteins correlated with pathogenicity and immunodominant antigens as vaccine candidate. Additionally, we conducted screening to find immune-cross-reactive proteins between *Salmonella* Enteritidis(SE) and *Salmonella* Typhimurium(ST) and SG.
Methods: Field isolates of SG, ST and SE used in this study were obtained from local poultry farms. SG OMPs were extracted by using 10mM HEPES buffer and 1% sarcosyl as described by Barenkamp. The extracted OMPs were subjected to 2-DE gel electrophoresis as described by O'Farrell's. Proteins loading in 2-DE was normalized by using Bradford assay. Next, spots were transferred to PVDF membrane and reacted with positive sera against SG, ST and SE. Excised spots were digested with trypsin andMatrix-Assisted Laser Desorption/Ionization - Time of Flight was measured protein quantities and identified.
Results and conclusions : Majority of SG-OMPs were located between 30-45kDa and these results are in accordance with previously reports on OMPs. Immunoblotting the membrane with SG polyclonal antibodies detected 29 spots. Out of 29 spots, 26 immunoreactive spots were identified using MALDI-TOF. ompA was noted as the most abundant protein among these immunoblot spots. The second high abundant protein noted was the chaperonin GroEL. In addition to these abundant proteins, many proteins belonging to different cellular pathways were identified in this study. Moreover, ompA and

012P (continued)
chareronin GroEL were identified in the cross-reactive screening between different serovars. These results suggest that chickens exposed to SE or ST during their lifetime are capable of mounting an immune response against subsequent SG infection. To conclude, this study identifies many SG immunogenic OMPs and provides information about the cross-protective antigens between salmonella serovars.

013P
Salmonella Enteritidis mutants with reduced Caco-2 cell invasiveness show impaired survival in chicken macrophages and reduced invasiveness in chicken liver cells.
H.-Y. Kim[1], D.H. Shah[2];
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Purpose: *Salmonella* Enteritidis (SE) is the number one cause of bacterial food-borne gastroenteritis world-wide. Contaminated poultry products such as meat and eggs are the primary sources of human infection. Unlike *S.* Typhimurium, the genetic basis of pathogenesis of SE infection in both human and chicken hosts is still poorly understood. We recently screened a transposon mutant library (n=4,992 mutants) of SE *in vitro* using invasion in well-differentiated human intestinal (Caco-2) epithelial cells as a surrogate marker for pathogenicity and identified several mutants with attenuated cell invasiveness. The objective of this study was to determine the pathogenicity of these mutants in chickens. Methods: A total of seventeen SE mutants were screened *in vitro* for their pathogenicity in chickens using invasiveness in chicken liver cells (LMH) and the uptake and survival within the chicken macrophages (HD-11) as a surrogate marker for their pathogenicity in chickens.
Results: There were no differences in the uptake of any of the mutant strains by avian macrophages. However, ten out of seventeen mutants showed significantly (P<0.05) reduced survival within the avian macrophages and reduced invasiveness in chicken liver cells when compared with the with the wild-type SE strain. These mutants had transposon insertion in genes encoding proteins of unknown functions (n=5), putative fimbrial adhesin (n=1), flagellar assembly protein (n=1), SPI-5 (n=1), SPI-1 (n=1) and a pseudogene (n=1). Mutants with transposon insertion in genes encoding curli fimbrial minor subunit (n=1) and hypothetical proteins (n=2) were attenuated in their invasiveness in LMH cells, but their survival in avian macrophages was not significantly affected.
Conclusions: This study demonstrates the role of several previously uncharacterized genes in the pathogenesis of SE infection in chicken. Further studies are ongoing to determine the pathogenicity of these mutants *in vivo* in orally challenged chickens and the role of the above genes in the survival of SE in egg albumen.

014P
Porcine circovirus ORF2 capsid protein express on the surface of a *Salmonella enterica typhimurium.*
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Purpose: Attenuated *Salmonella* vaccine vectors generally elicit strong antibody responses against the passenger antigen which is express on the bacterial surface. In this study we make ORF2 protein expressed *S. typhimurium* strain and which was tested immunogenecity of the both ORF2 and *S. typhimurium.*
Methods:Construct of surface display vector which is encoding ORF2 gene of PCV2 and then transfected in *S. typhimurium* H683 Δasd cells. Isopropyl-β-D-thio-galactoside(IPTG) is used as an inducer of ORF2 protein expression. Surface localization of ORF2 protein is certified by trypsin treatment test. The ability of the displayed antigen to induce an immune response was measured using PCV2-antibody ELISA analysis in guinea pig model. Antibody titer against *S. typhmurium* also is measured by ELISA assay. Protective efficacy is tested by challenge test with virulent *S.typhmurium.*
Results: PCV2 ORF2 protein which was linked to C-terminal of OmpA domain expressed on the surface of the *S. typhmurium.* SDS-PAGE and western blotting assay using ORF2 protein specific antibody show ORF2 protein is successfully expressed and localized on the outer membrane of the *S. typhmurium.* Surface localization of ORF2 protein can be certified using trypsin treatment assay. ORF2 protein band is decreased depend on the enzyme treatment time. ORF2 expressed *S. typhmurium* bacterial cells(10^9 cfu/ml) immunized to the mouse elect high titer of serum antibody against PCV2 as well as *S. typhmurium.* 80% of the mouse which is immunized with ORF2 expressed *S. typhmurium* bacterial cells(10^9 cfu/ml) alive against virulent *S. typhmurium* challenge.
Conclusions:The results of this study show two important perspectives to multivalent vaccine for PMWS or PCVAD disease prevention. ORF2 of PCV2 protein antigen successfully expressed on the non-pathogenic salmonella live vector and act as a good vaccine candidate.

015P
Infection of C57BL/6 mice by *Trypanosoma musculi*, modulates host immune responses during *Brucella abortus* co-colonization.
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Purpose: Brucellosis, which results in fetal abortions in domestic and wildlife populations, is a disease of major concern for the US and developing world. Currently in the US, Brucellosis is maintained in wildlife in the Greater Yellowstone Ecosystem although a moderately efficacious vaccine (RB51) exists for protecting domestic cattle herds. However, recent *Brucella* outbreaks are attributed to transmission from wildlife to cattle herds. In elk brucellosis develops into a chronic infection in which the pathogenic mechanisms facilitating a chronic infective state are unknown. Recently, evidence has been presented that *B. abortus* and *Trypanosoma cruzi* use homologous proteins to modulate the host immune response to enable survival *in vivo*. In Wyoming, elk are chronically infected with *Trypanosoma cervi*, which is suspected to modulate host responses in a similar manner to that of *T. cruzi*. Our study seeks to elicit whether a synergistic relationship exists between the two pathogens contributing to chronicity of *Brucella abortus* infection in elk.
Methods: To identify a synergistic relationship, we employed a murine brucellosis model, using *Trypanosoma musculi* as a simulant for *T. cervi*. Groups of C57BL/6 mice were infected with either 5 x 10^4 CFU *B. abortus* strain 19 (S19), 1 x 10^6 DF *T. musculi* or both. Sera were collected weekly and assayed for IgG, IgM, IL-10 and IFN-γ. Mice were also splenectomized to determine bacterial load near the end of normal brucellosis infection.
Results: Mice co-infected with *B. abortus* S19 and *T. musculi* had significantly decreased sera IFN-γ and increased IL-10 both at 7 and 14 dpi after inoculation with S19 ($p<0.05$). Splenic bacterial load was also found elevated in the co-infected group at 70 dpi ($p<0.05$). Additionally, mice in this group also had decreased IgM and IgG serum concentrations compared to controls ($p<0.05$).
Conclusions: These results suggest that immune modulatory events resulting in a decreased inflammatory response are occurring in the mouse during co-infection and that further experiments are warranted to determine if *T. cervi* similarly affects the course of *B. abortus* infection in elk, leading to persistence or chronicity of the pathogen *in vivo.*

BACTERIAL PATHOGENESIS POSTERS

016P
Comparison of abortion and infection after experimental challenge of pregnant bison and cattle with *Brucella abortus* strain 2308.
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Purpose: The purpose of the current study was to compare the pathogenesis of *Brucella abortus* in bison and cattle using a standard experimental challenge model.
Methods: A comparative study was conducted using data from naive bison (n=45) and cattle (n=46) from 8 and 6 studies, respectively, in which a standardized *Brucella abortus* strain 2308 experimental challenge was administered during midgestation. The incidence of abortion, fetal infection, uterine or mammary infection, or infection in maternal tissues after experimetnal challenge was greater (P<0.05) in bison as compared to cattle. In animals that did abort, the time between experimental challenge and abortion was shorter (P<0.05) for bison when compared to cattle. *Brucella* colonization of four target tissues, and serologic responses on the standard tube agglutination test at the time of abortion, did not differ (P>0.05) between cattle and bison.
Results: The results of our study suggest that naive bison and cattle have similarities and differences after experimental exposure to a virulent *B. abortus* strain.
Conclusions: Although our data suggests that bison may be more susceptible to infection with *Brucella*, some pathogenic characteristics of brucellosis were similar between bison and cattle.

017P
Evaluating the Biochemical Based Differentiation of *Bibersteinia trehalosi.*
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Iowa State University, Ames, IA, USA.

Purpose: The aim of the study was to properly identify *Biberstieinia trehalosi* based on phenotypic and biological characterization. Additionally, evaluation of the antimicrobial susceptibility patterns of *B. trehalosi* from bovine respiratory cases will be used to determine impact of antimicrobial resistance on pathogenicity.
Methods: Ten isolates from Iowa State University and 9 isolates from outside sources were obtained for use in this study. Ten preliminary identified *B. trehalosi* isolates obtained from the Iowa State University Veterinary Diagnostic Laboratory (VDL) and two others from outside labs were sequenced and identified with 16S analysis. Based on the 16s analysis, some were determined to be different bacteria: *Gallibacterium anatis* and *Mannheimia varigena*. Biological characterization and antimicrobial sensitivity was performed on the isolates based on the protocol of the VDL Bacteriology department at Iowa State University.
Results: All seven *B. trehalosi* isolates fermented trehalose sugar broth. Based on TREK gram negative identification (GNID) plate, the 7 *B. trehalosi* isolates did not consistently produce the same results. Antimicrobial sensitivity was performed on all isolates. Five of 14 isolates showed to be highly resistant to most antibiotics. One of 14 isolates had moderate resistance to about half of the antibiotics. Eight of 14 isolates were susceptible to most antibiotics.
Conclusions: Molecular characterization may be necessary to correctly identify *B. trehalosi* suspect isolates.

018P
Resolution of *Mycobacterium bovis* phylogeny using genome-wide single nucleotide polymorphisms.
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Purpose: Piecemeal analysis of *Mycobacterium bovis* (MBO) genomes and conventional genotyping methods have not lent to a comprehensive resolution of its genetic diversity to explain the wide range of disease phenotypes caused by this zoonotic pathogen. Conventional genotyping methods target a small hypervariable region on the genome of MBO and provide anonymous biallelic information insufficient to develop MBO phylogeny. We propose using genome-wide single nucleotide polymorphisms (SNPs) would have sufficient resolution to develop trait-allele interactions as evidenced in studies with *M. tuberculosis.*
Methods: Using the high throughput iPLEX^{TM} Massarray (Sequenom), we interrogated the MBO genome for 350 loci including geneic (n =306) and intergeneic (n =44) regions for SNPs. A collection of 77 MBO isolates associated with bovine bTB outbreaks in the US between 1990-2009 and isolated from a variety of mammalian hosts - cattle, deer, elk, elephant, swine, and humans were used for the study. 62 *M. tuberculosis* isolates from human, primates, birds, and elephants were also included in the analysis.
Results & Conclusions: Based on 206 variant SNPs between the MBO strains, five major clusters consistent with epidemiologic and other strain-typing information were identified. All the MTB isolates were identical at the 350 loci. This SNP based phylogeny provides new insights into the evolution of MBO and a gateway for studying strain genotype-disease phenotype correlations that we undertook in an *in vitro* infection model of the disease with 4 virulent MBO strains isolated from human, cattle (2) and deer and investigated them for their virulence based on survival in macrophages and relative gene expression profile of various virulence genes at different time points post-infection. The preliminary results reveal a differential survival of 4 strains; however relative gene expression for mce4C, PE6, speE, mmpL12 did not reveal a differential expression pattern; we are currently evaluating more targets to draw specific conclusions including SNPs affecting intergeneic loci.

019P
Lack of gut oxalate-degrading bacteria is a risk factor for calcium oxalate urolithiasis in cats.
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University of Minnesota, Saint Paul, MN, USA.

Purpose: Calcium oxalate urolithiasis is an increasingly serious problem in cats, but there are few treatment and prevention options. Therefore, our laboratory is investigating an oxalate-degrading intestinal bacterium *Oxalobacter formigenes*, whose strict requirement for oxalate has the potential to ameliorate hyperoxaluria by reducing enteric absorption of dietary oxalate. Enteric colonization of *Oxalobacter formigenes* is correlated with absence of hyperoxaluria and consequently calcium oxalate (CaOx) urolithiasis. The bacterium has been found previously in humans, cattle, sheep, rats, dogs and cats. Therefore, we hypothesized that the lack of *O. formigenes* is a risk factor for CaOx urolithiasis development in cats. A case-control study was conducted to test the hypothesis.
Methods: Fecal samples from 47 cats in two matched-cat groups, based on the same age, breed and gender, were collected with 24 samples from CaOx-affected cats and 23 samples from healthy cats without any history of stone disease. DNA was extracted from 200 mg of feline stool sample and *O.*

019P (continued)
formigenes presence was determined by quantitative PCR amplification of the *Oxc* gene.
Results: The presence of *Oxc* was higher in healthy non-stone forming cats than in the cats with CaOx stones, with 30.4 and 12.5 percent positive in healthy and affected cats, respectively. Quantitative differences were observed as well, with 40700±15400 (mean±SE) copies per gram of feces observed in healthy cats as compared to 4600±2640 (mean±SE) copies per gram in cats with hyperoxaluria. Also, the odds of having no CaOx stones is three times higher for cats with *Oxalobacter formigenes* present in the gut compared to those without the bacteria (OR=3.05 - 95% CI: 0.68, 13.74).
Conclusions: These results indicate that the lack of these bacteria increases the risk of increased urinary oxalate excretion and stone formation. Also, it makes the presence or absence of *O. formigenes* a relevant risk factor, and provides opportunities for development of novel and effective tools for prevention of CaOx urolithiasis.

020P
Bacterial community profiling of tonsils from diseased pigs using terminal restriction fragment length polymorphism analysis.
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Purpose: The last decade has seen culture-independent techniques, normally used in microbial ecology, being applied to human and animal body sites in order to profile the bacterial communities present. Much of this work is concerned with the role commensal (non-pathogenic) bacteria play in disease. Studies are being undertaken to characterize the bacterial community of the tonsil of the soft palate of swine. In addition to harbouring a large number of poorly-characterized commensal organisms, this tissue is known to be the reservoir of many primary and opportunistic pathogens.
Methods: As part of a larger study on risk-based surveillance of respiratory infections in growing pigs, tonsillar samples were obtained from unthrifty animals in closeout groups in finisher facilities. Routine microbiological analysis was performed and samples from both culture and tissue were characterized using T-RFLP analysis with the Phusion® Bacterial Profiling kit. T-RFLP electropherograms were produced by capillary electrophoresis and the fragment peak sizes obtained used for tentative bacterial identification by searching Finnzymes' and other databases. Once T-RFLP profiles have been generated, pyrosequencing will be performed from representative samples. DNA extraction methods and primer specificity have been optimized to ensure 'hard-to-lyse' bacterial DNA is being captured for analysis.
Results: Preliminary analysis of plate cultures shows variation of community patterns between farms and to a lesser extent, between individual pigs within farm. T-RFLP analysis looks to be more sensitive than routine microbiological analysis; however, putative identification of specific bacterial pathogens in most cases can be made only to the genus level.
Conclusions: Due to its quickness and less expensive advantages, T-RFLP analysis holds promise for obtaining a more complete picture of microbial communities than is currently available by routine bacterial culture methods.

021P
Regulation of Leptospira interrogans LenA protein.
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Purpose: Previous studies indicate that LenA is a multifunctional bacterial surface protein, and suggest it to be a virulence factor that is involved in adhesion, immune evasion, and invasion by pathogenic leptospires. LenA is expressed during mammalian infection and its transcription is induced at physiological osmolarity. How pathogenic leptospires regulate the expression of LenA is not known.
Methods: Electrophoretic mobility shift assays (EMSAs) were performed using a biotin-labeled probe and light shift chemiluminescence. The probe used for specific binding studies was a 296-bp fragment of the *Leptospira interrogans* serovar Lai *lenA* 5' noncoding DNA, which is highly conserved in all pathogenic strains. The same probe was used as bait in affinity chromatography to identify DNA-binding proteins in the lysate prepared from *L. interrogans* serovar Lai.
Results: Two proteins in the cytoplasmic extract of *L. interrogans* bound with high affinity to the biotinylated DNA affixed to the avidin-linked magnetic beads. These proteins were eluted off the beads, separated on SDS-PAGE, excised from gels and analyzed by matrix-assisted laser desorption ionization-time of flight (mass spectrometry). The ability of these newly identified leptospiral proteins to bind *L. interrogans* serovar Lai *lenA* 5' noncoding DNA was further assessed by EMSAs. Our results indicate that these novel leptospiral protein binds specific DNA sequences located immediately 5' of *lenA* locus. These are the first site-specific DNA-binding proteins to be identified in *L. interrogans*.
Conclusions: This study provides valuable insights into the regulation of an important virulence factor of *L . interrogans* and may provide target(s) for therapy.

022P
The embryo lethality of E*scherichia coli* isolates and its relationship to the presence of virulence-associated genes.
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The Embryo Lethality of *Escherichia coli* Isolates and Its Relationship to the Presence of Virulence-Associated Genes
Purpose: The aim of this study was statistically analyzed to determine if the chicken embryo lethality and the presence of nine virulence-associated genes were correlated with each other, and which gene is the best contribution to embryo lethality.
Methods: We, therefore, examined 58 E. coli strains isolated from visceral organs of chickens with colibacillosis for the presence of nine virulence genes (*fimC, tsh, fyuA, irp2, iucD, cvi/cva, iss, astA, and vat*) by polymerase chain reactions.
Results: *FimC* (Type I fimriae) was detected with the highest prevalence in 93.1% of the isolates, followed by followed by iucD (67.24%), iss (58.62%), tsh (34.48%), cvi/cva (34.48%), fyuA (32.76%), astA(31.0%), irp2(27.59%), and vat (17.24%). In the chicken embryo lethality assay, while causing various mortalities from 5% to 100%, most isolates (54/58; 93.1%) appeared in their ability as moderately or highly virulent to kill embryos over than 10% mortality. Statistical analysis exhibited that high positive correlations were observed between the presence of virulence genes and chicken embryo lethality. In addition, presence of the *iucD* (aerobactin) gene was the trait the best contribution to embryo mortality by using Multivariate Model.
Conclusions: Based on the results, it was suggested that the more frequently expressed the virulence genes are, the higher the mortality of the chicken embryo will be, and the gene that can best estimate mortality the embryo is *iucD.*

BACTERIAL PATHOGENESIS POSTERS

023P

Putative adhesins of *Actinobacillus suis*.

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Purpose: *Actinobacillus suis*, a common commensal in conventionally reared swine, has gained importance in recent years as an opportunistic pathogen of high health status herds. It can cause septicaemia, pleuropneumonia, and meningitis in animals of all ages and abortion in sows. Little is known about the pathogenesis of this organism including the critical first steps of host colonization. Therefore, the objective of this study was to identify putative adhesins recently sequenced *A. suis* H91-0380 genome.
Method: Bioinformatic analysis was done using tools such as BLASTx, BLASTn, and BLASTp available at http://www.ncbi.nlm.nih.gov/.
Result: BLASTx analysis revealed that most putative adhesins of *A. suis* were similar to those reported in other *Pasteurellaceae*. Two putative autotransporters (ATs) had homology only with *A. pleuropneumoniae*, 2 had highest homology with *A. ureae*, and the fifth putative AT had homology to *A. pleuropneumoniae*, *Pasteurellaceae*, and a number of distantly related organisms. Eleven putative outer membrane proteins (OMPs) were detected, all with high homology to *A. pleuropneumoniae* and other *Pasteurellaceae* including *A. ureae*, *A. minor*, *M. haemolytica* and *H. ducreyi*. Fourteen genes that appear to form a tight-adherence (*tad*) locus, associated with adherence and biofilm formation, and 4 genes predicted to form an *apf* operon, responsible for type IV pilus formation, were present in *A. suis* and were highly homologous to those in *A. pleuropneumoniae*. Three additional putative fimbrial adhesins were also identified, two of which had homologues in *A. pleuropneumoniae* strains and other *Pasteurellaceae*, and one that shared homology with *A. ureae* and other *Pasteurellaceae*. A homologue of ComEA, a competence gene also known to bind fibronectin, was also detected in the *A. suis* genome.
Conclusion: Of the 40 putative adhesins of *A. suis* identified in this study, only two did not have homologues in *A. pleuropneumoniae*, results which again suggest that observed differences in species and tissue tropism of these two organisms are likely due to fairly subtle differences. Work is currently underway to definitively characterize the role of these genes in attachment and invasion

024P

A case report describing detection of *Rhodoturola minuta* fungemia in an ewe lamb.

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Purpose: Description of *Rhodoturola* fungemia. Methods: An 8-mo-old crossbred ewe that was normal upon physical examination was humanely euthanized for tissue collection. Prior to euthanasia, whole blood was collected via jugular venipuncture into 60-ml syringes containing EDTA anticoagulant. After sacrifice, brain was removed and choroid plexus harvested. Internal organs appeared normal upon gross examination and one kidney was removed. Choroid plexus and kidney were immediately placed into sterile 50-ml tubes containing ice cold PBS and were transported to the laboratory for cell isolation. Monocytes were purified from whole blood by density gradient centrifugation, and cell suspensions were obtained from the choroid plexus and kidney by mincing the tissue and digestion in 0.25% trypsin. Each tissue-specific cell suspension was placed in a 75-cm^2 tissue culture flask in RPMI culture medium with antibiotic and fungizone, L-glutamine, and 5% FBS, and was cultured at 37°C, 5% CO_2 in a humidified atmosphere. After several weeks of culture, fungi began budding out of the choroid plexus. Shortly thereafter the budding was observed in the kidney cell cultures--and eventually--in the monocyte cultures as well. Cell monolayers of all lineages appeared healthy and after fungi removal and medium replacement continued to grow. Serum from the lamb was submitted to the Veterinary Diagnostic Laboratory at Colorado State University for fungal diagnosis and was found negative for *Aspergillus*, *Blastomyces*, *Coccidiodomycosis*, and *Histoplasmosis*. DNA was isolated from fungi collected from tissue culture supernatants, and used in a set of pan-fungal PCR assays with DNA from *Candida* acting as a positive control. PCR products were sequenced and BLAST analysis performed. Results: The unknown fungal sequence aligned with 100% identity to *Rhodotorula minuta*, an emerging opportunistic pathogen. Samples have been submitted to The Fungal Testing Laboratory at The University of Texas Health Science Center at San Antonio for additional validation. Conclusion: We believe this to be the first report of *Rhodoturola* fungemia in a sheep in the United States. USDA is an equal opportunity provider and employer.

COMPANION ANIMAL EPIDEMIOLOGY POSTERS

025P

Seroprevalence of pandemic H1N1 and seasonal human influenza in domestic cats in Ohio.

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Domestic cats have several features that make them ideal vehicles for interspecies transmission of influenza viruses; however, they have been largely overlooked as potential reservoir or bridging hosts. In this study we conducted serological surveillance to assess the prevalence of novel pandemic H1N1 as well as seasonal human influenza virus infections in domestic cats in Ohio,USA. Four-hundred serum samples collected from domestic cats (September 2009 - September 2010) were tested using hemagglutination inhibition (HI) test. Seroprevalence of pandemic H1N1, seasonal H1N1 and H3N2 were 22.5%, 33%, and 43.5% respectively. In addition, a significant association between clinical feline respiratory disease and influenza virus infection was documented. In this sample of cats, prevalence of pandemic H1N1 did not follow the seasonality pattern of seasonal H1N1 or H3N2 influenza, similar to observations in humans. Pandemic H1N1 seroprevalence did not vary in relation to ambient temperature changes, while the seroprevalence of seasonal H3N2 and H1N1 influenza viruses increased with the decline of ambient temperature. Our results highlight the high prevalence of influenza infection in domestic cats, a seasonality pattern comparable to that of influenza in humans, and an association of infection with clinical respiratory disease.

EPIDEMIOLOGY AND ANIMAL HEALTH ECONOMICS POSTERS

026P

The use of nucleocapsid N and nsp7 proteins in PRRS genotype 1 virus diagnostics.

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Purpose: The aim of this work was to express recombinant structural N and nonstructural Nsp7 proteins of Czech field strain (genotype I) of PRRS virus and to compare their diagnostic sensitivity and specificity. Another goal was to analyze the use of Nsp7 antigen for the differentiation of post-infection and post-vaccination antibodies in pigs vaccinated with an inactivated vaccine.
Methods: ORF 7 and Nsp7 genes were cloned into pDest17 vector. The expression of N and Nsp7 proteins was tested in different *Escherichia coli* strains. Resulting recombinant proteins were purified by IMAC using a polyhistidine tag under denaturing conditions from the insoluble fraction of bacterial lysate. Purified N and Nsp7 proteins were applied as antigens in indirect ELISA tests. Serological reactivity of both proteins was assessed on a panel of 274 swine sera separated to three groups: 1) 44 sera from non-vaccinated herds free of PRRS infection for calculation the cut-off value, 2) 44 PRRS-negative pigs vaccinated by the inactivated vaccine and 3) 186 serum samples from PRRS positive farms for a comparison of both ELISA tests.
Results: Both antigens proved to be suitable for serological detection of PRRS specific antibodies, showing diagnostic specificity of 95.6 %, and sensitivity of 90.5 % for N protein ELISA test and 85.7 % specificity and 97.2 % sensitivity for Nsp7 ELISA test. Nsp7 antigen proved to be suitable for differentiation of post-infection and post-vaccination antibodies. Sera from PRRS free herds vaccinated by inactivated vaccine were compared with N and Nsp7 based ELISA tests. Although 100 % of sera gave positive results
in N protein based ELISA test, these sera were tested negative with Nsp7
antigen.
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027P

Molecular epidemiology of highly pathogenic porcine respiratory and reproductive syndrome (hp-prrs) variants in southeast asian countries (Vietnam, Laos and Cambodia).

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Porcine respiratory and reproductive syndrome (PRRS) is an economically important infectious disease in the swine industry all over the world. The causative virus is a member of the Arteriviridae family in the order of Nidovirales. In 2006, a highly pathogenic PRRS (HP-PRRSV) outbreak occurred in China killing approximately 2 million heads of pig. The progeny variants of this outbreak not only continue to affect Chinese swine industry from 2006 till now, but the virus has spread across border to several Southeast Asian (SEA) countries including Vietnam, Laos, Cambodia, Thailand and Myanmar. In Vietnam, the highly pathogenic PRRS was first recognized in the northern part of the country in 2007 and later led to a countrywide epidemic affecting at least 65,000 pigs. The subsequence epidemic of HP-PRRS also started in the northern Vietnam in April 2010, and the virus rapidly spread southward in Vietnam (July 2010). HP-PRRS outbreaks were also confirmed in Laos PDR (June 2010) and in Cambodia (July 2010). To characterize the epidemiology of these PRRSV outbreaks in Southeast Asia, we sequenced 211 PRRSVs from Southeast Asia and incorporated them into current Chinese HP-PRRSV genome diversity database. Majority of sampling (n=187) were originated from a single introduction, which subsequent spread throughout the three SEA countries including Vietnam, Cambodia and Laos. Other SEA PRRSV sequences formed small clusters in the tree that are probably originated from several independent introductions. However, epidemiological impact remained unknown. In addition, our analysis indicated that the spreads of HP-PRRS among SEA countries are relatively frequent, probably due to connections of the pig industries in these countries. In summary, since the HP-PRRSV outbreak in China, the virus has been introduced more than three times into southeast Asia, followed by inter-country transmission of virus that amplified the intensify of their impact.

EPIDEMIOLOGY AND ANIMAL HEALTH ECONOMICS POSTERS

028P

Proposal for ongoing targeted surveillance in the PRRS area regional control and elimination (PRRS ARC&E) projects.

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Purpose: Targeted surveillance has been recognized as one approach that can improve the efficiency of surveillance. The most straightforward application of the approach is when a veterinary authority is tasked with the goal of providing evidence of freedom from infection. Surveillance activities are also important for endemic production diseases, but the framework within which the surveillance occurs is much different than for diseases exotic to this population. A particular challenge is the approach to surveillance during porcine reproductive and respiratory syndrome virus (PRRSv) area regional control and elimination projects (ARC&E). This is challenging because the surveillance system should be able to: (i) declare freedom from infection in herds that have successfully eliminated infection, (ii) identify new infections in the area, particularly if they occur in herds that have a high risk of transmitting the infection, and (iii) monitor disease trends over time. Fulfilling these objectives under conditions of scarce resources requires a strategic and structured approach regarding the inclusion of herds for ongoing surveillance, flexibility in accepting the results of different testing protocols, and the choice of appropriate testing procedures for specific tasks.
Methods: For the ongoing surveillance in the Niagara PRRS ARC&E we have proposed the implementation of a "high risk - high consequence" approach. The approach requires the implementation of a short survey that can be applied to all herd types. The questionnaire assesses basic external biosecurity practices, willingness to eliminate the virus, and consequences of spread. A "surveillance algorithm" has been proposed regarding the incorporation of the most recent diagnostic information, external biosecurity level, potential infectiousness of herds, and phase of elimination into a targeted surveillance approach.
Results: Twenty eight "high risk - high consequence" herds within the Niagara PRRS ARC&E project have been selected for inclusion in the overall surveillance activity.
Conclusions: Recent developments in the area of surveillance methodology should be considered for regional disease control.

029P

Outbreak investigation of neonatal piglet's diarrhea disease in a county of China.

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Purpose: An increase in mortality of neonatal piglets occurred between the 1 and 15 April 2011 in a pig farm in China. The course of disease was rapid and more than 20% neonatal piglets dead with scouring in 2-3 days. The index farm and other farms in the country were investigated on 18th and 19th April. This study aimed to describe the outbreak and analyze associated risk factors initially.
Methods: A case is a litter of neonatal piglets where morbidity related to diarrhea was greater than 10% in a litter and mortality of cases increased rapidly to at least 50% within in the county in 2011. Case finding was conducted using questionnaires to collect epidemiological data. Descriptive and analytical statistics were performed. Laboratory samples were inconsistently submitted by farmers for testing including post-mortem examination, bacterial culture, virus isolation prior to the investigation. Initial differential diagnosis considered included Porcine Reproductive and Respiratory Syndrome (PRRS), Pseudorabies and Transmissible Gastroenteritis of Pigs (TGE).
Results: On the index farm, 5-10% of litters had diarrhea and mortality of cases less than 10% during the past 12 months prior to outbreak. Thirty-three percent (7/21) of pig farms and 26% (94/362) of litters in all investigated farms in the country demonstrated high mortality in piglets under 10 days of age between 1 March to 15 of April 2011. Initial analysis indicates that first parity sows were most associated with highest mortality in piglets (OR=20.0; 95% CI: 7.7-53.9). TGE virus was isolated from 3 of 10 samples from the index farm. Results for PRRS and Pseudorabies were negative. Post-mortem results from the index farm were also consistent with TGE. None of the pig farms in this county conducted TGE vaccination in 2010.
Conclusions: These data suggested that TGEV and is a cause of this outbreak. Further, systematic study is needed to assess herd immunity and the type of co-infections circulating in the county pig population in order to assist with future prevention and control of diarrhea in neonatal pigs.

030P

Investigation of *Bacillus anthracis* Outbreak in the Central Part of Myanmar in 2010.

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Anthrax is an endemic disease of cattle in Myanmar and the Livestock Breeding and Veterinary Department (LBVD) promotes vaccination for disease control and prevention. However, the disease outbreaks occur sporadically in central Myanmar. In September 2010, LBVD received information of a suspected anthrax outbreak in humans in Myingyan province, Mandalay State, located in central Myanmar. In response to this outbreak, LBVD conducted an outbreak investigation. The objectives of this investigation were to confirm the outbreak of anthrax in humans and animals, to identify the source of human infection and to identify magnitude of the outbreak in animals to inform further preventive and control measures. Due to security reasons and terrain, animal case finding was conducted in only 11 villages in the affected area between 7 to 14 of September 2010. A participatory approach was used including focus group interviews with livestock owners, traders, village chiefs, slaughter men and human cases. Face-to-face interviews were conducted with 275 interviewees to retrospectively identify potential animal cases in all villages. There were 28 human anthrax cases hospitalized since September 2010 and skin lesion was evident in all cases. Human cases were linked with consumption or contact with dead animals. There were several unhygienic practices noted during the investigation, such as handling of the infected carcasses with bare hands and discarding carcasses into the river. Six out of 11 villages had a recent history of animal anthrax cases and anthrax infection was confirmed by bacterial culture in one animal that died suddenly. Cattle in the study area had never received anthrax vaccination. In addition, cattle sharing the pasture area were exposed to cattle that recently died. Flooding between June to August may also be associated with this outbreak. From this investigation, we confirmed an outbreak of anthrax in animals likely associated with human infections. Humans exposed to animals require further education on how to protect themselves and their animals from anthrax.

031P

Pathobiological Analysis of Bovine Viral Diarrhea Virus Identified in the Republic of Korea.

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Purpose: Bovine viral diarrhea virus (BVDV) is distributed worldwide in cattle herds and leads to a significant economic impact on cattle producers. BVDV infections have been associated with a variety of clinical manifestations from unapparent or mild to severe acute and fatal mucosal disease. The

031P (continued)
BVDV infection is widely spread among Korean indigenous cattle. Therefore, we report the genetic and pathobiological features of BVDV-2 isolates recently identified in the Republic of Korea (ROK).
Methods: Five outbreaks from 5 different farms in Gyeongbuk province, ROK were presented. The outbreaks occurred in the winter of 2010 and only calves between 20 and 40 days-old were affected. Clinical signs were characterized by depression, anorexia, hemorrhagic diarrhea, weakness, and respiratory disorders. 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: These five outbreaks were tested and confirmed positive for BVDV by RT-PCR. Phylogenetic and nucleotide analysis revealed that severe acute bovine viral diarrhea virus (BVDV) outbreaks from Korean indigenous calves belonged to BVDV-2a, and were identical to those of the highly pathogenic BVDV-2 strain 890, with identical virulence markers and classified as highly virulent.
Conclusions: These results support the fact that the association of BVDV-2 with severe disease indicates the presence of highly virulent isolates in the ROK. The identification of the highly virulent BVDV-2 isolates among Korean indigenous calves may have important implications for epidemiological studies, diagnostic and immunization strategies.

032P
Brucellosis, an important Porcine Reproductive problem in Bangladesh.
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Out of 590 million pigs in the world, about 34% are raised in tropical countries. In Bangladesh, pig rearing continues to be primitive because they are raised by certain rural people who are educationally, economically and socially most backward. But now-a-days, there are some livestock related projects working with rural ultra poor people to eradicate extreme poverty. These projects are funded by European Union and implemented in disaster prone areas of Bangladesh. The objective & purpose of the project is to improve food security and nutritional well-being of the ultra poor people through pig rearing. In study areas, there are some tribal communities like Cobbler, Sing, Orang, Saotal, Pahan, Bormon, Robidas etc. are rearing pigs by receiving asset grant from the project fund. The primary purpose of pig rearing is the meat production including pork, bacon, fat or ham, production of skin, bristles and manure used as fertilizer either for soil of for fish-ponds. So it has a great value to identify brucellosis in pigs because of socioeconomic impacts of rural ultra poor people. A total of 146 swine were screened by Rose Bengal Test (RBT) using *Brucella abortus* and *Brucella melitensis* antigen. Positive, doubtful and negative samples were further confirmed with indirect Enzyme Linked Immunosorbent Assay (I-ELISA) and all the indirect ELISA negative samples were confirmed as negative. A structured questionnaire was used to collect epidemiological information of the animals. The overall serological prevalence derived 4.79% by RBT (*B. melitensis*) and 2.55% by I-ELISA. An insignificant higher prevalence was observed in male than that of female (4.0%). Significant association was found between abortion and occurrence of brucellosis. Relatively higher prevalence of brucellosis was found in swine of 3rd or more parity.

033P
A prevalence survey of bovine trichomoniasis in Mato Grosso do Sul, Brazil.
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Purpose: Trichomoniasis, caused by the protozoal parasite *Tritrichomonas foetus*, is a bovine reproductive disease of importance worldwide due to economic losses from infertility, early embryonic death, and extended calving intervals. The disease is transmitted sexually from asymptomatic carrier bulls to susceptible cows and heifers during natural breeding. We are interested in discovering the prevalence of trichomoniasis in the state of Mato Grosso do Sul (MS), Brazil, where the beef industry has an important economic role.
Methods: We conducted a pilot study sampling 34 mature bulls from three farms in the southern Pantanal region of MS, Brazil. Smegma samples were collected with a pipette by vigorously scraping the epithelium of the prepucial fornix while applying suction. After collection, the samples were transferred immediately to InPouch™ TF media test kits for incubation. The TF pouches were examined daily for 7 days post-collection with a light microscope for the characteristic movement of *T.foetus* within the media.
Results: The presence of the pathogen *T. foetus* was undetected using the InPouch™ TF test kits in all 34 bulls sampled. We are currently awaiting the results of PCR in order to confirm our negative findings.
Conclusions: In the future we hope to expand the number of bulls tested and include a wider region of the state in order to gather more comprehensive data on the prevalence of Trichomoniasis in Mato Grosso do Sul, Brazil.

034P
M. hyosynoviae and M. hyorhinis: a retrospective study.
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Infectious arthritis is well recognized by its major role in causing lameness in pigs regardless the stage of production, and among all pathogens *M. hyosynoviae* and *M. hyorhinis* are mainly known by causing arthritis in swine from weaning to finish. Although there is no recent epidemiological information available, it seems that their role as a cause arthritis has increased in recent years in swine systems within the U.S. Midwest. In order to get better insights from the clinical/diagnosis perspective, an evaluation of the Veterinary Diagnostic Laboratory-ISU database from 2003 to 2010 was carried out by pulling out all cases associated with lameness, and sub-categorizing it by its cause according to the diagnostician overview. Overall, 47% of the clinical cases associated with lameness were caused by infectious arthritis, and 53% of the cases were had a no definitive diagnosis, or were associated with a nutrition deficiency. In average from all the years, 17% of the arthritis cases were caused by Mycoplasmas; however, the frequency of cases diagnosed with Mycoplasmas arthritis has increased over the past few years, reaching 37% of the total of arthritis cases in 2010. *M. hyorhinis* has been more frequently in animals younger than 10 weeks of age, but on the other hand, *M. hyosynoviae* associated arthritis cases has occurred more often in animals older than 10 weeks, representing in average 37% and 68% of the total number of cases over all the years, consecutively. In summary, Mycoplasmas diagnosis has increased over time, but neither prevalence nor perspectives in regards of disease association in the field can be drawn, because of the unstable number of samples being submitted to the VDL, lack of standardization in terms of sample collection or animal selection in the field, usage of antibiotics against other pathogens which may interfere with the viability of microorganisms associated with arthritis, and yet to be developed and/or validated other assays such as IHC and qPCR for a better approach on different specimens selected for diagnosis.

FOOD AND ENVIRONMENTAL SAFETY POSTERS

035P

Minimizing antibiotic resistance transmission: How are health goals and protocols communicated between workers and management on dairy farms?

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Purpose: Prudent use education to minimize antimicrobial resistance on dairy farms has primarily targeted owner-operators. However, as dairy farms increase in size, the gap between work associated with prudent use and owners widens. Language and culture variation and task specialization are the norm on large dairy farms and create on-farm communication challenges. The study objective was to describe dairy farm communication strategies for developing goals and accomplishing daily calf care.
Methods: Surveys were designed to describe the flow of on-farm information and information sources for informing decisions. Questions, first written in English then translated into Spanish, focused on calf feeding and health monitoring. For both of these we addressed communication surrounding goals and protocol development and daily routines. All surveys were conducted in-person in English or Spanish based on respondent's preference. Our goal was to target large herds (>500 milking cows) and compare communication patterns to small herds.
Results: Surveys were conducted in 5 states (WA, OR, ID, AZ, and NY) on 32 large and 14 small herds. We report on data from WA. We conducted 3-5 face-to-face interviews per farm (veterinarians, owners, managers, and calf caregivers); 2-3 of which were in Spanish. In general, owners and herd managers are very involved in goal setting and consult veterinarians, nutritionists, and middle management as they set goals. Calf managers tend to be bilingual (Spanish and English) and communicate daily with Spanish-speaking calf caregivers (feeders and treaters) on workflow, resolving unexpected issues, and meeting goals. Calf management changes and goal development reside with owners, but they seek input from calf managers and from calf caregivers, with input from the latter usually being relayed via calf managers.
Conclusions: Middle management bridges the communication gap between owners and the dairy farm workforce; consequently, investing in their people management and communication skills and calf care knowledge would be beneficial. However, educational programs targeting all individuals involved in calf care decisions are likely needed to effect change.

036P

Epidemiology of Shiga toxin-producing Escherichia coli (STEC) in swine.

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Purpose: More than 100,000 cases of illness are estimated to be attributed to Shiga toxin-producing Escherichia coli (STEC) annually in the United States. STEC cause hemorrhagic colitis (HC) and hemolytic uremic syndrome (HUS) in humans. Many STEC outbreaks are associated with food contaminated by animal feces. Some human cases of STEC O157:H7 and non-O157 infections have been associated with pork products. To this date, little is known about swine STEC. We have conducted a prospective cohort study to achieve our objectives: to provide the first longitudinal descriptive epidemiology of STEC shedding in US swine during the finishing period and to characterize swine STEC isolates.
Methods: Three cohorts of pigs from one production company were included. In each cohort, 50 pigs were randomly selected and individually identified. Fecal samples were collected from each pig once every two weeks, throughout the 16 weeks of the finishing period (eight samples/pig). Samples were assayed for STEC detection by enrichment (10 min in TSB, pH 3 followed by incubation for 15 h in modified TSB at 42 °C) followed by the polymerase chain reaction (PCR) targeting the Shiga toxin genes and the intimin protein gene (eae). Shiga toxin gene-positive enrichments were plated onto Rainbow Agar O157, ChromAgar O157, and ChromAgar STEC. Presumptive STEC isolates were recovered, confirmed by PCR, and serotyped.
Results: At the time of submission, presumptive STEC isolates were recovered from 37 of 50 pigs in the first cohort and from 12 of 50 pigs in the second cohort. Further confirmation by PCR and serotyping results are pending. Collection in the third cohort will start in fall 2011.
Conclusions: These data will be critical to fill current gaps in swine STEC epidemiology and the association between STEC in swine, pork, and humans.

037P

Effects of corn-based distillers grain (dg) inclusion into feeding rations on the burden of *Escherichia coli* O157:H7 incommercial feedlot settings.

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Purpose: Use of corn-based distillers' grain has been associated with an increased prevalence of *Escherichia coli* O157:H7 in feedlot cattle. However, previous research has been conducted in small-pen research settings and the relevance to real-world commercial settings is unclear. Our objective was to quantify the potential relationship between the use of distillers' grain co-products and *E. coli* O157:H7 pathogen load in real-world feedlot settings.
Methods*:* Ten commercial feedlots were split into cohorts in which finishing diets contained either high DG (>15%) or low DG (< 8%). Feedlots were sampled on 4 occasions from June thru October of 2010 with approximately 6 weeks between each sample collection. At each feedlot visit, 4 pens of cattle within 3 weeks of harvest were enrolled and 24 freshly voided fecal pats were collected from each pen. Ten gram samples were placed into 90mL of modified tryptic soy broth supplemented with novobiocin and enriched for 14 hours at 42°C. Enrichments were subjected to immunomagnetic separation, plated onto CHROMagar™ with novobiocin, and incubated for 18 hours at 37°C. Suspect-colonies were confirmed via latex agglutination.
Results*:* Of 3,840 samples, *Escherichia coli* O157:H7 was recovered from 16.7% of the samples. Controlling for within-feedlot and within-pen clustering, adjusted prevalence was 14.3%, indicating some evidence of within-group clustering. Variation within feedlots over time was substantial; for example, within one feedlot, mean sample-level prevalence ranged from 7.6% to 42.7%. Prevalence within the 4 sampling rounds was 19.9% (sample window=28JUN to 12JUL), 21.0% (AUG), 14.1% (SEP) and 11.7% (OCT). Significant interaction was observed between DG use and sampling round ($P<0.01$). Prevalence among high use was greater than those with low use for sampling rounds 1, 3 and 4 but not different for round 2. Averaged across time, prevalence among high and low users was 21.4% versus 7.3%, respectively.
Conclusions: These data indicate a higher prevalence of *Escherichia coli* O157:H7 associated with increased inclusion rates of distillers' grain into feeding rations in commercial feedlot settings.

038P

Prevalence of Shiga toxin-producing *Escherichia coli* serogroup O26 in feces of feedlot cattle.

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Purpose: Our objectives were to describe the prevalence, distribution, and virulence genes of Shiga toxin-producing *E. coli* (STEC) serogroup O26, and describe relationships between O26 and O157 prevalence in feces of feedlot cattle.
Methods: Feces were collected from cohorts of feedlot cattle over an 11-week period, subjected to immunomagnetic separation (IMS) for O26, and plated

038P (continued)
on MacConkey agar. Isolation of O157 was performed using both IMS and direct plating methods. Ten pooled colonies from O26 IMS plates and single colonies from O157 plates were tested with a multiplex PCR that detects STEC O-serogroups and virulence genes (*stx*1, *stx*2, *eae*A, *hly*A).
Results: Results indicate the sample-level prevalence of O26 was 22.7% overall, and within cattle cohorts the weekly prevalence ranged between 0 and 50.8%; all cohorts tested positive more than once. Prevalence of O26 appeared to vary over time, with a seemingly higher prevalence in late June/July as compared to May/early June; a similar temporal pattern was observed for O157 results. Shiga toxin genes were commonly detected within samples, but not for all samples positive for O26. The prevalence of O26 appeared to be higher in fecal samples positive for O157 than those negative for O157.
Conclusions: Preliminary conclusions are that O26 are common, but with a variable prevalence, in cohorts of feedlot cattle. Furthermore, fecal prevalence of O26 and presence of virulence genes may be less than what was observed for serogroup O157. However, the fecal prevalence of O26 may have a similar temporal distribution in feedlot cattle as observed for O157.

039P
Prevalence, serotyping, and antimicrobial resistance profiling of *Salmonella* isolated from conventional and organic Oklahoma retail ground poultry.
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Purpose: Contaminated poultry meat is one of the main foodborne sources of *Salmonella*. The purpose of this study was to determine the prevalence, serotypes, and antimicrobial resisitance profiling of *Salmonella* in Oklahoma retail ground poultry. Methods: A total of 199 samples of retail ground poultry meat (150 ground Turkey and 49 ground chicken) were collected from various stores of six major supermarket chains in Tulsa, Oklahoma. Of the collected 199 meat samples, 141 were conventional and 58 were organic representing various poultry brands. Poultry samples were subjected Conventional *Salmonella* isolation. Antimicrobial resistance were screened using the agar dilution method and serotyping was performed at the NVSL. Results: Eighty-two of the tested 199 samples (41%) were positive for *Salmonella*. *Salmonella* was recovered from sixty six (46%) of the 141 conventional samples and sixteen (27%) of the 58 organic ones. Fourteen (28%) chicken samples and 69 (45%) turkey samples were positive for *Salmonella*. Twenty seven *Salmonella* isolates were selected for serotyping and antimicrobial resistance profiling. Seven serotypes were determined among the isolates; Anatum_ver_15+ (1), Saintpaul (4), Tennessee (9), Enteritides (3), Anatum (3), Senftenberg (4), and Ouakam (2). Sixteen different antibiotic resistance profiles were determined among the twenty seven screened *Salmonella* isolates with all isolates showing resistance to at least four antimicrobials. A dendrogram generated by the BioNumerics software linked some serotypes to particular antibiotic resistance profiles. All isolates were susceptible to Amikacin, Chloramphenicol, Ceftriaxone, Ceprofloxacin, and Trimethoprim/Sulfamethoxazole. Resistance to the rest of the 16 tested antimicrobials was as follow; Gentamycin (100%), Ceftiofur (100%), Streptomycin (93%), Amoxicillin/Clavulanic acid (96%), Kanamycin (89%), Cephalothin (85%), Ampicillin (85%), Tetracycline (70%), Sulfisoxazole (30%), Cefoxitin (19%), Nalidixic (15%). Conclusions: Multidrug resistant *Salmonella* was recovered from retail ground turkey sold in Oklahoma with slightly higher prevalence in conventional samples.

040P
Loxoribine pretreatment reduces *Salmonella enteritidis* organ invasion in day-old chickens.
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Purpose: Neonatal poultry exhibit a transient susceptibility to infectious diseases during the first week of life that leads to significant economic losses and stems from an impairment of innate and acquired defense mechanisms. Consequently, ways to stimulate or modulate the natural immune response is emerging as an area of interest for food animal producers including the poultry industry. The objective of this study was to determine if pretreatment of day-old chicks with Loxoribine, a TLR7 agonist and immune modulator, protects young chicks from *Salmonella enteritidis* organ invasion or *S. enteritidis*-induced mortality. Methods: Loxoribine (0-100µg) was administered intra-abdominally (IA) to day-old broiler chicks and 4 h later the birds were challenged orally with *S. enteritidis*. Twenty-four h post-challenge, birds were euthanized and the liver and spleen removed and cultured for the presence of *S. enteritidis*. This was carried out on 3 separate occasions using 26-50 chicks/dose/experiment. Results: Pre-treatment of chicks with Loxoribine (6.25-25µg) significantly reduced liver and spleen organ invasion by *S. enteritidis*. Higher doses (50-100µg) of Loxoribine had no effect or rendered the birds more susceptible to organ invasion by *S. enteritidis*. A second challenge model was also evaluated (IA Loxoribine followed by an IA *S. enteritidis* challenge) to determine if Loxoribine pretreatment (0-100µg) protected against *S. enteritidis*-induced mortality. This experimental model was conducted twice with 35-50 chicks/dose/experiment. Regardless of dose, Loxoribine did not offer protection and, in fact, increased susceptibility to *S. enteritidis*-induced mortality. Conclusions: The results showed broiler chicks pre-treated with Loxoribine, a potent TLR7 agonist and immunomodulator, were protected against organ invasion by *S. enteritidis* but not from *S. enteritidis*-induced mortality. The results obtained in this study indicate there is a potential application for using Loxoribine to increase protection of young chicks when they are most susceptible to infections with *Salmonella* and that additional administration routes, doses, and timing should be evaluated.

041P
Antimicrobial resistance in the ten most common S*almonella* serotypes at the S*almonella* bank at Washington State University, Pullman: 1986-2010.
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Epidemiologic and ecologic analyses of microbial isolate databanks of pathogens important to public and animal health are useful in understanding when, where and why antimicrobial resistance is generated. We analyzed historical *Salmonella* antimicrobial resistance data from 1986 and 2010 to determine antimicrobial resistance diversity in the ten most common serovars found in humans, cattle and avian species. Isolates were from bovine and avian clinical samples sent to WA Animal Disease Diagnostic lab, human clinical isolates collected by WA State Department of Health, and non-clinical cattle isolates collected through research projects. The dataset was accessed on April 26, 2011 and contained 22,058 *Salmonella* isolates. We selected the ten most common serotypes found in humans, cattle and avian species that were tested for susceptibility to eight antimicrobials: (ampicillin 10 µg, chloramphenicol 30 µg, gentamicin 10 µg, kanamycin 30 µg, streptomycin 10µg, triple-sulfa 300 µg (or sulfisoxazole 250 µg), tetracycline 10 µg, and trimethoprim-sulfa 1.25/23.75 µg.) using Kirby-Bauer disk diffusion method. After selecting the 10 common serovars and excluding isolates that lacked information on year collected, source and location, 7750 isolates were available for analysis. Isolates were reclassified as resistant or susceptible and the antimicrobial resistance phenotype was determined based on eight antimicrobials. Simpson's and Shannon's diversity indices were calculated for each serotype and species. Cluster analysis was used to group isolates with similar AMR phenotypes. Multinomial logistic regression was used to establish association between phenotype and serotype. There were 102 unique antimicrobial resistance phenotypes and 43.3% were pan-susceptible. Resistance to multiple drugs was common (ACSSuT 14.7%, AKSSuT 8.35%, ACKSSuT 5.3%). Antimicrobial phenotype diversity was highest in S. Dublin followed by S. Typhimurium, S. Heidelberg and S. Newport; and higher in bovine compared to human isolates. Despite source and temporal similarities, there is a strong serovar impact on the diversity of resistance phenotypes.

FOOD AND ENVIRONMENTAL SAFETY POSTERS

042P

Detection of *Salmonella typhimurium* using dna barcode amplification assay.

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Purpose: The DNA barcode amplification assay was employed for detecting *Salmonella typhimurium*, which is one of the most common cause of foodborne illness.
Methods: The DNA barcode assay depends on a sandwich conformation based on specific biological interaction of magnetic beads with gold nanoparticles (AuNPs) harboring DNA barcode. The magnetic beads hold the probe DNA for target recognition. The AuNPs possess the oligonucleotides in which DNA barcode and target recognition sequences are integrated. The target analyte (*S. typhimurium* genomic DNA) can connect the magnetic beads with AuNPs.
Results: To evaluate the performance of this protocol, the thiol-modified oligonucleotides and biotinylated probe DNA were immobilized onto the AuNPs (13 nm in diameter) and the streptavidin-labelled magnetic beads, respectively. Following the lysis of food samples such as *S. typhimurium*-contaminated milk, the magnetic beads and AuNPs were mixed with the lysed samples. The magnetic beads-AuNPs complex was formed through the target DNA in the presence of *S. typhimurium* in milk, magnet-separated, and subsequently PCR-amplified in high sensitive manner.
Conclusions: The DNA barcode-based biosensor coupled with PCR was developed, providing an excellent selective and sensitive performance.

043P

Prevalence of and antimicrobial resistance in E. coli and Salmonella isolated from raw chicken nuggets at retail purchased in Canada.

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In 2009, Salmonella Enteritidis and Salmonella Heidelberg accounted for two of the top three human clinical Salmonella serovars isolated from patients in Canada. Resistance to antimicrobials of very high importance to human health, as designated by Health Canada were seen in these serovars, more so in S. Heidelberg. The consumption of chicken nuggets has been an identified risk factor of S. Heidelberg infection in Canada. The Canadian Integrated Program for Antimicrobial Resistance Surveillance (CIPARS) monitors the prevalence and resistance of enteric bacteria in beef, chicken legs or wings and pork meat at retail; CIPARS has not, to date, included breaded processed chicken products, which have been implicated in some recent Salmonella outbreaks. Since February 2011, the CIPARS retail sampling infrastructure has been used to collect raw chicken nuggets. 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, CMV2AGNF. Salmonella was recovered from 41% (45/111) samples. The top three serovars recovered were S. Enteritidis (16/45), S. Heidelberg (14/45), S. Kentucky (11/45). Multi-drug resistance was seen in all three top serovars (6/45), with resistance to ceftiofur in two isolates each of S. Heidelberg and S. Kentucky. Recovery of E. coli was 83% (92/111). Resistance to ciprofloxacin was not observed in either Salmonella or E. coli. Resistance to antimicrobials of very high importance to human medicine such as ceftiofur (19/92), ceftriaxone (21/92) and amoxicillin-clavulanic acid (22/92) was found (always in combination with ampicillin and one or more cephalosporins). The prevalence of resistance found E. coli isolates in Canadian sold raw chicken nuggets closely reflects CIPARS retail chicken surveillance figures. The presence of possible ESBLs and cephamycinases-producers in both Salmonella and E. coli emphasizes the importance of prudent use of antimicrobials in poultry production in the context of dissemination of antimicrobial resistance and public health.

044P

Virulence and antimicrobial resistance profiling of *Staphylococcus aureus* including MRSA strains isolated from retail meats sold in Oklahoma.

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Purpose: *Staphylococcus aureus* is one of the main causative agents of life threatening infections both in humans and animals. The purpose of this study was to characterize the virulence and antimicrobial susceptibility of *Staphylococcus* and MRSA strains isolated from Oklahoma retail meats. Methods: A total of 564 retail meat samples were purchased from several grocery stores in Tulsa, Oklahoma. *Staphylococcus aureus* strains were isolated from the retail meats by enrichment and plating on Baird-Parker agar medium. Antimicrobial resistance profiling for 16 antimicrobial was performed using the agar dilution method. The presence of 18 toxin genes was detected by PCR. Results: A total of 230 out of 564 (40%) meat samples were positive for *Staphylococcus aureus*. The prevalence of *Staphylococcus aureus* in the different meat types was as follows; pork 943%), beef (18%), beef livers (80%), chicken livers (18%), chicken gizzards (22%), chicken (42%), and turkey (64%). Twelve MRSA strains were recovered from two chicken samples. Antimicrobial resistance to 16 antibiotics was tested for 614 *Staphylococcus aureus* and 12 MRSA recovered isolates. Resistance to the tested antimicrobials was as follows; Ampicillin (96%), Penicillin (61%), Tetracycline (57%), Doxycycline (48%), Azithromycin (39%), Erythromycin (39%), Oxacillin (33%), Kanamycin (28%), Vancomycin (27%), Cefoxitin (25%), Ciprofloxacin (24%), Gentamicin (24%), Rifampin (16%), Clindamycine (14%), Trimethoprm/Sulfamethazale (13%), and Chloramphenicol (10%). All recovered isolates were tested for the presence of 18 *Staphylococcus aureus toxin genes*. Panton-Valentine leucocidin (PVL) genes were prevalent in MRSA isolates. The prevalence of hemolysins was high (*hla (88%), hld* (88%), and *hlB (52%)*). The prevalence of enterotoxins ranged from 0.3% to 17.2% and no genes for exfoliative toxins were found. Conclusions: Multidrug resistant *Staphylococcus aureus* is highly prevalent in retail meats sold in Oklahoma particularly poultry and beef livers. The recovered strains, even the non MRSA ones, carry a number of variable toxin genes.

045P

Detection of methicillin-resistant *Staphylococcus aureus* in swine oral fluids.

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Introduction: Methicillin-resistant *Staphylococcus aureus* (MRSA) is a global health concern and has been isolated in foods of animal origin. MRSA sequence type 398 (ST 398) appears to be found with increased frequency from pigs. Oral fluids are an efficient, cost-effective method of collecting diagnostic samples from pigs. Purpose: 1) Determine level of detection of MRSA in swine oral fluids, 2) Compare detection of MRSA from environmental and oral fluid samples from same swine facilities, 3) Assess prevalence of MRSA in oral fluid samples submitted for other routine diagnostic testing. Methods: All oral fluids were collected using cotton ropes. Environmental samples were collected using sponges. Samples were processed with enrichment in broth (10g tryptone/L, 75g NaCl/L, 10g mannitol/L and 2.5g yeast extract/L and incubated at 35º C for 24 hrs) and streaked onto chromogenic media (BioRad MRSASelect) and incubated for 24-48 hrs at 35º C. Suspect colonies were streaked for purity on Sheep's blood agar and further characterized with biochemical testing, oxacillin screen with disc diffusion, PBP 2a latex testing, *mec*A and PVL PCR, and spa typing. "Clean" swine oral fluids were spiked and serially diluted (10^8 to 10^{-2} CFU/ml) separately with four strains of MRSA (ATCC 43300, spa types t548, t034, t002). Environmental and oral fluid samples were collected during same visit to swine facilities and tested for MRSA. Aliquots of oral fluids were taken from diagnostic samples

045P (continued)
submitted for routine testing and tested for MRSA. Results: MRSA was detected as low as 1CFU/ml from spiked samples. MRSA was detected in 6.97% (3/43) of oral fluids collected from 12 swine facilities. No MRSA was detected from 51 environmental samples tested from the same facilities. MRSA was detected in 5.88% (24/408) oral fluid samples submitted for other routine diagnostic testing. Spa types found in swine oral fluids include: t002, t111, and t2876. Conclusion: The use of oral fluids appears to be a sensitive, efficient and cost-effective method for detecting MRSA in swine.

046P
Effect of electron beam irradiation on *Mycobacterium avium* complex in ground pork.
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Purpose: To obtain information on the effect of electron beam irradiation on the viability of *Mycobacterium avium* complex serovars 1, 2, 4, 8 and 9 in ground pork.
Methods: *Mycobacterium avium* complex serovars 1, 2, 4, 8 and 9 isolated from swine lymph nodes cultured in Middlebrook's 7H9 Liquid Medium at 37°C for 7 days were added to ground pork and exposed to 0.75, or to 4kGy using electron beam irradiation.
Results: *Mycobacterium avium* complex serovars 1, 2, 4, 8 and 9 were killed at 4kGY; however each of the serovars survived irradiation at 0.75kGy.
Conclusions: Electron beam irradiation at 4kGy killed each of 2 strains of *M. avium* complex serovars 1, 2, 4, 8 and 9 in ground pork.

047P
Comparative characteristics of listeria monocytogenes serotype 4b biological properties.
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Purpose: It is now one of the essential points in the study of food of animal origin regulated by international standards, is to identify agents poisoning, especially Listeria. The aim of this work was to study the comparative of the biological properties of Listeria monocytogenes strains cerotype 4b, isolated in Ukraine and Poland.
Methods: The studies were conducted molecular biological, cultural-morphological, biochemical, hemolytic methods; antibiotic sensitivity.
Results: The results of molecular biological studies (PCR) confirmed the identity of the isolated strains to the species Listeria monocytogenes; strains fermented with production of acid without gas glucose, maltose, rhamnose; and not fermented xylose. Eskulin, A-manozidaze, D-arabital, rhamnose, methyl- A-D-glucopyranoside tests were positive. The strains showed ß-hemolytic activity on the blood agar; in the CAMP-test near crop growth Staphilococcus aureus formed a small area of ß-hemolysis. All tested strains showed sensitivity to penicillin, cephalosporins, aminoglycosides, tetracyclines, macrolides, lincosamides, fluoroquinolones. Strains isolated in Poland in most cases revealed more resistant to penicillins, aminoglycosides, macrolides, cephalosporins, third generation. To cephalosporins of the 1st and 2nd generation, tetracyclines, lincosamides, fluoroquinolones observed individual variability in the sensitivity of the strains.
Conclusions: Research in this area gives a possibility to identify patterns of changes in populations of microorganisms, and identify mechanisms of antibiotic resistance in bacteria.

GASTROENTERIC DISEASES POSTERS

048P

Effect of dietary zinc supplementation (dietary ZnO and microencapsulated zinc oxide) on colibacillosis in weaned pigs.

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Purpose: The purpose of this study was to investigate whether microencapsulated zinc oxide(100ppm) was able to defend colibacillosis and *E. coli* as apramycin and high dose of dietary ZnO (3000ppm) and then compare their effects.
Methods: In this experiment, 36 piglets were divided into 6 groups, and each group was allocated with 6 weaned pigs. Total experimental period was 14 days. Group B, C, and D were fed with feed additives (apramycin =10g/kg, ZnO =30g/kg, shield zinc =10g/kg). Group E and F were fed with feed additive(CTC zinc =2.5g/kg). Group A, B, C, D, and E were inoculated with *E.coli* K88 on the 7th day (3ml, 1x10^{10} CFU/ml). Group F was not inoculated with *E.coli* K88. Temperature, average daily gain (ADG), feed conversion ratio (FCR), and diarrhea score were checked daily. Diarrhea index was graded according to fecal consistency (0 =normal; 1 =abnormal feces but not diarrheic; 2 =mild diarrhea; 3 =severe watery diarrhea). Blood samples were collected on the 1st, 7th, 9th, 11th, and 14th day for ELISA. Fecal samples were collected on the 7th, 9th, and 14th day. Organ samples(duodenum, jejunum, ileum, colon, cecum, and mesentery lymph node) were collected for detection of *E.coli* K88 and quantitative analysis by real-time PCR. H&E stain was used in the small intestine for measuring villi height : crypt depth and number of goblet cells.
Results: ADG and FCR was improved in bacteriophage group. In diarrhea, the result of bacteriophage group was improved. In ELISA, Group A had higher OD value than other groups($p<0.05$). In quantitative analysis, concentration of Group A was higher than those of other groups in feces and organs ($p<0.05$). Groups fed with zinc oxide products had no significant difference. In V/C ratio (villi height to crypt depth ratio), Group F had high V/C ratio compare with other groups ($p<0.05$). Groups fed with microencapsulated zinc oxide had higher ratios than other groups.
Conclusions: Overall, this study clearly showed that supplementation ofmicroencapsulated zinc oxides suppressed the expression of PWD in pigs inoculated with enterotoxigenic *E. coli*. Bacteriophage is expected to prevent and treat colibacillosis.

049P

The effect of bacteriophage on weaned pigs challenged with *Clostridium perfringens* type A and C.

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Purpose: *Clostridium* (*C.*) *perfringens* can cause necrotic enteritis by bowel-irritating toxins in weaned pigs. Recently, several workers have investigated bacteriophage's effect to reduce antibiotic drugs in feed. The objective of this study was to investigate the effects of clostridium bacteriophage on weaned pigs.
Methods: 24 piglets were divided into 4 experimental groups, and each group was allocated with 6 weaned pigs. Group B and D were fed with *Clostridium* bacteriophage (1x10^{9}PFU/g, CTCBIO, Korea) orally via feed (1g/kg) from the 1st day of experiment. Group C and D were inoculated with *C. perfringens* type A and C individually on the 7th day (1x10^{10} CFU/ml, 1.5+1.5ml). The experiment period was 14 days. Growth performance and clinical signs including body temperature and diarrhea index were recorded. Diarrhea index was graded according to fecal consistency (0 =normal; 1 = abnormal feces but not diarrheic; 2 = mild diarrhea; 3 = severe watery diarrhea) by Sherman's methods (2). Fecal samples (5g) were collected on the 7th, 9th, and 14th day for quantitative analysis by real time PCR. All pigs were necropsied to measure pH of stomach, ileum, and colon by litmus paper (ADVANTEC, Canada) and to detect
pathogens by real-time PCR.
Results: Body temperature and ADG were imporved in bacteriophage group. Diarrhea index of Group C was always higher than that of Group D during the experimental period. The pHs of the stomach, ileum, and colon of Group C and D were higher than those of the other groups. In Group D, the pH of ileum was lower than that of Group C ($p<0.05$). In quantitative analysis, α-toxin and β-toxin of Group C samples were higher than those of Group D in feces and organs ($p<0.05$).
Conclusions: In this study, the groups fed with *Clostridium* bacteriophage showed
significantly better growth performance, reduced clinical signs (body
temperature and diarrhea index), and reduced detection of *C. perfringens*
than not-fed groups. The results suggest that *C. perfrigens* bacteriophage can contribute to reducing clostridal enteritis incidence and relieving clinical signs in weaned pig.

050P

The effect of bacteriophage on weaned pigs challenged with *Escherichia coli.*

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Purpose: *Escherichia* (*E.*) *coli* is the major cause of diarrhea for newborn and post-weaning pigs and is responsible for significant losses in farms worldwide. The objective of this study was to prove using bacteriophage is effective for weaned pigs infected with *E. coli*.
Methods: Piglets were divided into 4 groups, and each group was allocated with 6 weaned pigs. Group A and B were not inoculated with *E. coli*, and Group C and D were inoculated with *E. coli* (3ml, 1x10^{10} CFU/ml). Group B and D were fed with feed additive (1mg feed additive per 1kg feed) which include bacteriophage(bacteriophage titer: 10^{9} PFU/g, CTCBIO, Korea). The total experimental period was 14 days. Bacteriophage was fed to Group B and D daily from the 1st day of experiment, and challenging with *E. coli* (*E. coli* K88 =1.5ml, *E. coli* K99=1.5ml) was conducted on the 7th day. Body temperature, growth performance (ADG, FCR), and diarrhea index were checked daily. Diarrhea index was graded according to fecal consistency (0, normal; 1, mild; 2, moderate; 3, watery). After necropsy, pHs of gastrointestinal tracts (stomach, small intestine, and large intestine) were checked. Tissue samples (duodenum, jejunum, ileum, colon, cecum, and mesentery lymph node) were collected for detection of *E .coli* K88 and K99, and quantitative analysis was conducted by real-time PCR.
Results: In body temperature, the results of Group C and D were higher than those of other groups. The growth performance was improved in bacteriophage group. In diarrhea score, the result of Group C was higher than that of Group D. In pH of the ileum and colon, Group C was higher than Group D($p<0.05$). In quantitative analysis of *E. coli* K88, the ileum, colon, and mesentery lymph node of Group C showed high concentrations compare with Group D ($p<0.05$). In quantitative analysis of *E. coli* K99, colon and mesentery lymph node of Group C show high concentrations compare with Group D ($p<0.05$).
Conclusions: In this study, the results of pigs fed with diet supplements containing bacteriophage were better than those of pigs fed with diet supplements containing no bacteriophage. Thus, supplementation of *E. coli* bacteriophage is effective against *E. coli* infection.

GASTROENTERIC DISEASES POSTERS

051P

The effect of bacteriophage on weaned pigs challenged with *Salmonella typhimurium.*

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Purpose: Salmonellosis has caused heavy losses in swine industry and is a challenge to the global swine industry because of its implications for public health. The objective of this experiment was to evaluate effects of bacteriophage in weaned pigs infected with *Salmonella (S.) typhimurium.*
Methods: In this experiment, piglets were divided into 4 groups, and each group was allocated with 6 weaned pigs. Group B and C were fed with bacteriophage ($1x10^8$ PFU/g, CTCBIO, Korea). Group C and D were inoculated with 5ml of *S. typhimurium*($1x10^{10}$ CFU/ml) per head of piglet on the 7^{th}day. Body temperature, growth performance (ADG, FCR), and diarrhea index were checked daily. Diarrhea score was graded according to fecal consistency (0=normal; 1 = abnormal feces but not diarrheic; 2 = mild diarrhea; 3 = severe watery diarrhea). Blood samples were collected at 1^{st}, 7^{th}, 9^{th}, 11^{th}, and 14^{th} day for ELISA and PCR. Fecal samples were collected on the 7^{th}, 9^{th} and 14^{th} day. Organ samples (liver, duodenum, jejunum, ileum, colon, cecum, and mesentery lymph node) were collected for detection of *S. typhimurium* and quantitative analysis by real-time PCR(Measuring of V/C ratio (Villi height : crypt depth ratio) and number of goblet cells in intestines was used with H&E stain.
Results: In body temperature, Group C was apparently higher than other groups on the 3^{rd} day of post-inoculation ($p<0.05$). In growth performance and diarrhea index, group D were improved comparing with group. In quantitative analysis, Group C was higher than Group D in fecal sample, serum, duodenum, jejunum, and mesentery lymph node ($p<0.05$). In antibody repose, there was no significant difference between Group C and D ($p<0.05$). In V/C ratio, Group C was lower than that of Group D ($p<0.05$) whereas, in number of goblet cell, the opposite result was shown.
Conclusions: In this experiment, feeding supplemented with bacteriophage to piglets infected with *S. typhimurium* is effective to remarkably improve growth performance and reduce clinical signs and the concentration of antigen by real time PCR in duodenum, jejunum, mesentery lymph node, feces, and serum. Thus salmonella-bacteriophage is effective against samonellosis.

052P

The effect of bacteriophage on weaned pigs challenged with *Escherichia coli.*

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Purpose: *Escherichia* (*E.*) *coli* is the major cause of diarrhea for newborn and post-weaning pigs and is responsible for significant losses in farms worldwide. The objective of this study was to prove using bacteriophage is effective for weaned pigs infected with *E. coli*.
Methods: In this experiment, piglets were divided into 5 groups, and each group was allocated
with 7 weaned pigs. Group C, D and E were fed with bacteriophage ($1x10^8$ PFU/g, CJ, Korea). Group B, C, D and E were inoculated with 5ml of *E. coli*($1x10^{10}$ CFU/ml) per head of piglet via oral on the 7^{th} day. Body temperature, growth performance (ADG, FCR), and diarrhea index were checked daily. Diarrhea index was graded according to fecal consistency (0 =normal; 1 = abnormal feces but not diarrheic; 2 = mild diarrhea; 3 = severe watery diarrhea). Blood samples were collected on the 7^{st}, 9^{th}, 12^{th} and 14^{th} day for ELISA and PCR. Fecal samples were collected on the 7^{th}, 9^{th}, 12^{th} and 14^{th} day. Gastrointestinal ph was checked at fundus, duodenum, mid-jejnum, colon and cecum. Organ samples(duodenum, jejunum, ileum, colon, cecum, and mesentery lymph node) were collected for detection of *E. coli* and quantitative analysis by real-time PCR. Measuring of V/C ratio (Villi height : crypt depth ratio) in small intestines was used with H&E stain.
Results: In growth performance and diarrhea index, the results of C and E group were improved comparing with those of B group(Table 1, Figure 1). In gastrointestinal ph, the results of Group B were higher than other groups(Figure 2). In quantitative analysis, The results of Group C and E were higher than that of Group B in Ilium and mesentery lymph node ($p<0.05$). But there was no significance in other tissue samples(Figure 3). In fecal sample, Group C and E were higher than Group B. In antibody repose, Group C and E were higher than Group B. In V/C ratio, Group C and E was higher than Group E ($p<0.05$).

053P

Detection of rotavirus genotypes and coronavirus among diarrheic and healthy beef and dairy calves from Buenos Aires Province, Argentina.

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Purpose: Bovine rotavirus (BRV) and coronavirus (BCoV) are enteric pathogens associated with neonatal calf diarrhea (NCD). BRV is classified into G and P genotypes based on genetic variation in the outer capsid proteins VP7 and VP4, respectively. The aim of this study was to assess BRV and BCoV as etiologies associated with NCD in beef and dairy farms and to compare the genetic diversity of BRV strains circulating in the farms under study.
Methods:The survey was conducted in beef (n: 14) and dairy (n: 18) farms from Buenos Aires province, Argentina (2007-2011). Feces (beef n: 182; dairy n: 240) were collected from 2 to 60 day-old diarrheic (beef n: 102; dairy n: 137) and non-diarrheic (beef n: 80; dairy n: 103) calves. BRV and BCoV were detected by ELISAs. BRV positive samples were analyzed by heminested multiplex RT-PCR to determine the G (including G6 lineages III or IV, G8 and G10) and P genotypes (P[1], P[5] and P[11]).
Results: BRV was detected in 12 beef (86%) and in 15 dairy (83%) farms, while BCoV was detected in only 3 dairy farms (17%). BRV single infection rates were significantly higher ($p= 0.009$) in beef than in dairy calves (22.5% vs. 12.9%) and also in diarrheic (beef: 33 [32.3 %], dairy: 26 [19%]) than in non-diarrheic (beef: 8 [10%], dairy: 5 [4.8%]) ($p< 0.001$) calves. BCoV single infection rate in diarrheic dairy calves (2; 1.4%) was lower than that for BRV (26; 19%). Co-infections with both viruses were detected in 3 (2.2 %) calves. The 33 BRV strains from beef calves were genotyped as: G6IVP[5] (91%), (G6IV+G10)P[11] (3%), and G?(nontypeable)P[5] (6%). The 33 BRV strains from dairy calves were: G6IIIP[11] (42.4%), G6IVP[5] (9.1%), G10P[11] (9.1%), (G6III+G10)P[11] (6.1%), (G6IV + G10)P[11] (6.1%), (G6IV+G10)P[5+11] (6.1%), (G6IV+G10)P[5] (3%), G6IVP[5+11] (3 %), G8P[11] (3%), G?P[5] and G?P[11] (12%).
Conclusions: BRV was associated with NCD, either in beef or dairy farms, although the infection rate was higher in beef calves. The distribution of G and P genotypes differed according to the husbandry system with greater diversity in dairy calves as previously described in Argentina. In addition this work reports the first detection of a single infection by G8P[11] in dairy calves in Argentina.

054P

Detection and molecular characterization of bovine noroviruses among bovine diarrhea cases in US Midwest.

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Bovine norovirus (BNoV) is a member of genus *Norovirus*, family *Caliciviridae*. While the role of BNoV in enteric disease remains unclear, NoV is a major etiology of non-bacterial acute gastritis in human. Genetic similarity between human and animal NoVs has raised a zoonotic concern over the virus.

054P (continued)
To get better understanding of BNoV status in the field, 102 fecal samples were obtained in 2010 through diagnostic submissions from diarrheic animals in the US Midwest representing 82 different cattle herds in 8 different states and were tested by a real-time RT-PCR. Sequencing was conducted to characterize the phylogenetic relationship among BNoVs based on RNA-dependent-RNA polymerase (RdRp) in comparison to previously reported viruses. BNoV was detected in 53 samples (52%), indicating its endemicity in US cattle populations which requires further evaluation of its clinical significance. About 74% of the positive samples were from calves at less than 1 month of age. Among 38 BNoVs successfully sequenced for RdRp, 14 and 14 BNoVs were phylogenteically classified into GIII-1 and GIII-2, respectively. Sequence divergence between the 2 genotypes was 19-24%, while sequence identity within each genotype was 82-100% (GIII-1) and 91-100% (GIII-2), respectively. The remaining 10 BNoVs were closer to GIII-2 with 86-90% identities but were phylogenetically on a branch separated from the other GIII-2 BNoVs, suggesting that they might comprise a new genotype or subtype within GIII. Besides genetic diversity shown to exist among BNoVs, it is of particular interest that GIII-1 BNoVs were identified at a much higher rate than anticipated based on previous reports in US, although significance of the observation remains to be further studied. In conclusion, BNoV is widely distributed among diarrheic calves in the US Midwest and genetically diverse.

055P
Evaluation of a commercial rapid antigen detection kit for bovine enteric pathogens.
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Recently a commercial antigen-capturing ELISA kit in form of a dipstick (Entreicheck, Biovet®) was made available to bovine practitioners and producers for the rapid detection of bovine coronavirus (BCoV), bovine rotavirus (BRV), *Escherichia coli* (*E. coli*) K99$^+$, and *Cryptosporidium parvum* (*C. parvum*) in feces from diarrheic calves. The diagnostic performance of Entricheck was evaluated in comparison with a multiplex real-time polymerase chain reaction (mrtPCR) assay. One hundred fecal samples were procured from diagnostic submissions to the Iowa State University Veterinary Diagnostic Laboratory and were used for the assessment. The agreement (kappa value) in results for each pathogen between Enterichek and mrtPCR assay were 0.095 (BCoV), 0.521 (BRV), 0.823 (*E. coli* K99$^+$), and 0.840 (*C. parvum*). In comparison to mrtPCR assay, the diagnostic sensitivity of Enterichek was 60.0%, 42.3%, 71.4% and 81.5%; and the diagnostic specificity was 51.4%, 100%, 100%, and 98.6% for BCV, BRV, *E. coli* K99$^+$, and *C. parvum*, respectively. In conclusion, the kit could be a rapid test tool in the field for detection of *C. parvum* or *E. coli* K99$^+$ in feces from calves at acute stage of clinical disease. However, BCV-positive and BRV-negative results by the kit may need to be interpreted with caution due to their relatively low specificity or sensitivity under the conditions presented in the study.

056P
Secretion of heat-labile enterotoxin by porcine-origin enterotoxigenic *Escherichia coli* strains.
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Purpose: The potential for porcine enterotoxigenic *E. coli* (ETEC) isolates to secrete heat-labile enterotoxin (LT) is poorly documented. This study was done to determine whether porcine ETEC strains secrete LT and if so, to compare the concentrations of secreted toxin in different culture media.
Methods: Seventeen *E. coli* strains were cultured in 3 different media: human *w*ild-type (WT) LT+ H10407, 5 porcine WT LT+, 4 porcine WT LT-, and 7 recombinant (4 LT+, 3 LT-). Media included: i) BBL brain heart infusion (BHI) with 2% Casamino acids and lincomycin (90 µg/mL), pH 7.0, (0.3% glucose); ii) Bacto Casamino acids plus Difco yeast extract (CAYE), pH 7.5 (CAYE-L); and iii) CAYE plus glucose (0.25% final concentration), pH 8.5 (CAYE-M). Each strain was cultured in 15 mL of each media in a 125 mL conical flask for 18 h at 37ºC and 225 rpm. Following culture and centrifugation, supernatant fluids were collected, filtered (0.2 µm) and assayed for the presence of LT by GM1-ELISA. LT concentrations (ng/mL) were estimated by standard curves based on OD levels with purified LT in each plate. Three independent experiments were conducted per strain and media type.
Results: All LT+ porcine WT strains and recombinant LT+ strains secreted the toxin into the culture supernatant. The concentrations of LT in the culture supernatants varied more among porcine WT strains when grown in BHI than in CAYE-L or CAYE-M. All LT+ porcine strains (WT and recombinant) secreted significantly less LT than did strain H10407 (P<0.001). Porcine WT parent and LT+ recombinant derivative strains showed no significant differences in their levels secretion (P>0.05). However, porcine-origin recombinant strains had significantly higher levels of secretion than did a DH5α LT+ clone (P<0.05).
Conclusions: This study demonstrated that porcine WT strains secrete LT, albeit to a lesser level than the high LT-producing prototype human WT strain H10407. The level of LT secretion of porcine WT strains may vary depending on the growth media used. Porcine WT strains and LT+ recombinant porcine strains secreted higher levels of LT than did a DH5α LT+ clone, apparently because of a more efficient type II secretion system.

057P
A longitudinal study of *Campylobacter jejuni* infection in beef cattle and their environment.
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Campylobacter jejuni is most commonly associated with contamination of poultry. However, recent studies have indicated beef cattle as another potential source of *C. jejuni* in the food chain. Cattle may become infected upon exposure to a contaminated environment or natural wild hosts. A cohort of thirty-six randomly selected cattle was followed from the ranch to feedlot, and harvest to quantify *C. jejuni* colonization. Feedlot environmental samples (including drag swabs, feed bunk swipes, water units, flies, and wild birds) were collected to describe *C. jejuni* feedlot contamination and potential infection sources. For carcass sampling, two sites were selected; the USDA-FSIS sampling area, and the ventral midline area. At slaughter, all cattle fecal samples were positive for *C. jejuni*, compared to one animal (3%) at the range and on feedlot arrival. Bird fecal samples collected at pre-arrival, months three and six showed that birds were consistently infected - 32%, 28% and 30%, respectively. A fly sample at pre-arrival also tested positive for *C. jejuni*. There was significant disagreement between isolation of *C. jejuni* from carcass swipes at USDA-FSIS versus ventral-midline sites at evisceration (P = 0.020): *C. jejuni* was isolated from the ventral midline area from 22 (61%) carcasses, compared to 13 (36%) carcasses from the USDA FSIS area. Most probable number (MPN) values at evisceration were significantly (P=0.014) and positively correlated (rs=0.407). Environmental sampling results indicate that wild birds and flies may be a source of contamination in beef cattle. Further characterization of the environmental and bovine isolates might identify which specific strains of *C. jejuni* are responsible for the subsequent infection of cattle. The ventral midline may be a useful addition to standard sampling areas to monitor *C. jejuni* contamination. Although cattle can become rapidly colonized with *C. jejuni*, following standard aging practices contamination of the meat is low.

GASTROENTERIC DISEASES POSTERS

058P

Short-term oral passive immunotherapy using anti-*Campylobacter jejuni* IgY does not reduce *C. jejuni* colonization from the intestinal tract of broiler chickens.

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Purpose: The onjective of this study was to produce chicken egg-yolk derived immunoglobulin (IgY) against five *Campylobacter jejuni* colonization associated proteins (CAPs) and evaluate their efficacy to reduce cecal colonization by *C. jejuni* in experimentally infected broiler chickens.
Methods: Recombinant *C. jejuni* CAPs including CadF, CmeC, FlaA, FlpA and MOMP were expressed in *E. coli* and purified by affinity chromatography. Recombinant CAPs were then used as antigens to hyperimmunize SPF laying hens to induce production of egg-yolk immunoglobulin (IgY). The antibody response in serum and egg-yolk was tested by indirect-ELISA. Egg-yolks were collected from immunized and non-immunized hens and lyophilized to obtain egg-yolk powder (EYP) with or without anti-*C. jejuni* CAP-specific IgY. Three-day old chickens were orally challenged with *C. jejuni* strain F38011 (10^8 CFU/bird). At 9 days
post-infection (i.e. 12 days of age), hyperimmunized egg-yolk powder (HEYP) containing IgY against each *C. jejuni* CAP, nonimmunized egg-yolk powder (NEYP), and a cocktail of five HEYPs were included in feed at 10% (w/w) concentration. Positive control and negative control group was given standard feed. Treated feed was provided *ad libitum* for three days starting from 12 days until the end of the experiment at day fifteen. Cecal samples were collected from each chicken at the end of experiment to enumerate *C. jejuni* counts..
Results: Anti-*C. jejuni* CAP-specific IgY levels were significantly higher in the serum samples and egg-yolk powders obtained from immunized hens as compared with the non-immunized hens. However, incorporation of IgY in the form of lyophilized egg-yolk powder in feed had no significant effect (P =0.47) on levels of cecal colonization by *C. jejuni* when compared with infection positive control.Conclusions: Short-term (3 days) oral passive immunotherapy using anti-*Campylobacter jejuni* CAP-specific IgY is not sufficient to reduce *C. jejuni* colonization from the intestinal tract of broiler chickens. Further studies are required to test if the long term therapy may be more *C. jejuni* colonization.

IMMUNOLOGY POSTERS

059P

Swine toolkit progress for the us veterinary immune reagent network.

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The US Veterinary Immune Reagent Network (US VIRN, www.vetimm.org) was established to address the lack of immunological reagents specific for veterinary species. Efforts are targeted at swine, ruminants, poultry, equine and aquaculture species. Our goal is to produce reagents that function in ELISA, Luminex assays, ELISPOT and flow cytometric applications. In the last year recombinant chemokines (CCL3L1, CCL4 CCL5 and CCL20) and cytokines [interleukin-6 (IL-6) and IL-22] were expressed in *Pichia*, purified and all but IL-22 shown to be bioactive using chemotaxis, upregulation of marker expression or cell stimulation assays. We have also cloned, expressed and proven bioactivity of swine immunoregulatory cytokines, IL-17A and IL-17F. Hybridoma fusions for monoclonal antibodies (mAb) to CCL3L1, IL-6, IL-13, IL-17A, interferon-alpha (IFNa) and IFNb are underway at Univ. Massachusetts. A sensitive fluorescent microsphere, Luminex bead, immunoassay for CCL2 was developed with US VIRN produced mAb and included in the 8-plex swine cytokine assay. At Cornell Univ. a fusion protein expression system was used to generate material for immunizations for swine T cell receptors, TCRαβ; hybridoma fusions are continuing. Additional fusions will target IFNAR, CD19, and NK cell markers, NKp36 (NCR3) and NKp44 (NCR2). The US VIRN website www.vetimm.org has a progress update for swine as are all bioassay methods and gene sequences. Since many swine cytokine and CD reagents are available commercially the website includes a listing of those reagents and their sources. Products developed in this proposal are available to collaborators and have been made commercially available through 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.

060P

Innate immune responses of porcine intestinal epithelial cells (IPEC-J2) to virus-associated virulence determinants.

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The specific goal of this study was to define the changes in the expression of genes involved in intestinal innate immunity to virus-associated molecular patterns. We stimulated porcine intestinal epithelial cells (IPEC-J2) for 3 and 24 hours with imiquimod, polyinosinic: polycytidylic acid (Poly I:C) and Poly IC with Lyovec. Cells with media alone were used as negative control. Cell lysates were processed for total RNA extraction and total RNA was used to prepare cDNA using reverse transcriptase reagents. The gene expressions for cytokines, TLRs, and RLRs (RIG-1 and MDA-5) were quantified using real time RT-PCR relative to cyclophillin-A as housekeeping gene. The expression of TLR-3, TLR-7 and TLR-9 genes did not change at 3 hrs and 24 hrs under any condition. TLR-4,-5,-6, and -8 were down regulated at 24 hrs by imiquimod and poly IC. TLR-2 was up regulated at 3 hrs by Poly IC with Lyovec. RIG-1 gene expression was up regulated by imiquimod and poly IC at 3 hrs but down regulated at 24 hrs by imiquimod. Poly IC and poly IC with Lyovec up regulated MDA-5 gene expression at 3 hrs. Out of various cytokines tested, the expression of anti-viral cytokine interferon-alpha was up regulated at 3 hrs but not significantly and remained steady at 24 hrs in response to imiquimod,. Interferon-beta was up regulated but not significantly at 3 hrs in response to all three viral ligands. Pro-inflammatory cytokines IL-1alpha was up regulated by imiquimod at 3 hrs and IL-1beta was down regulated by Poly IC and imiquimod at 24 hrs. Chemokine IL-8 was up regulated at 3 hrs.TNF-alpha was up regulated by Poly IC at 3 hrs and IL-12p40 was up regulated by imiquimod at 3 hrs. Antimicrobial peptides beta-defensin-1 was up regulated at 24 hrs by imiquimod and Poly IC and beta-defensin-2 was up regulated at 3 hrs by all three viral ligands. From these results, it is expected that IPEC-J2 cell line may serve as a good cell culture model to study the viral ligand interactions and possibly the virus pathogenesis.

IMMUNOLOGY POSTERS

061P

Serum neutralizing antibody titer is associated with PRRSV vaccination-induced protection against homologous and heterologous challenge.

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Porcine reproductive and respiratory syndrome (PRRS) is a high-consequence animal disease worldwide. The objective of this study was to identify immune responses that are predictive of protection against heterologous PRRSV challenge. Using MLV IngelVac PRRSV vaccine, its parental strain VR2332, and K-LI (a Kansas isolate of PRRSV), we compared immune responses induced by a single vaccination and by experimental infection of two different PRRSV isolates. Four groups (5 pigs/group) of pigs were utilized in this study in which groups 1 & 2 were vaccinated with MLV PRRSV and groups 3 & 4 were not vaccinated. Twenty eight days post vaccination (DPV), pigs in groups 1 & 3 were challenged with VR2332, and groups 2 & 4 were challenged with K-LI. All pigs were euthanized 14 days post challenge (DPC). PRRSV was identified by RT-PCR in serum samples from vaccinated pigs 7 DPV. Quantitative PCR analysis showed that PRRSV was detected in serum samples of groups 2, 3, & 4 pigs but not in group 1 pigs 7 DPC. Lung pathology score of group 1 pigs was significantly lower than that of groups 2, 3, & 4 pigs 14 DPC, confirming the vaccination-induced homologous protection. Although PRRSV-specific antibody in the serum can be detected 7 DPV, serum neutralizing antibody against VR2332 was detected only after 28 DPV. Serum neutralizing antibody against K-LI was detected only in group 2 pigs 14 DPC. ELISpot assay indicated that VR2332 is a stronger inducer of IFN-γ-secreting PBMCs than K-LI in all pigs, and group 2 pigs possessed more PRRSV-specific IFN-γ-secreting PBMCs. This study indicates that heterologous challenge induces a higher level of cellular immune response against PRRSV in vaccinated pigs, but only serum neutralizing antibody titer is associated with PRRSV vaccination-induced protection against homologous and heterologous challenge.

062P

Evaluation of a real-time PCR assay for pseudorabies virus surveillance purposes.

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Purpose: Pseudorabies virus (PRV) is of significant economic importance for the swine industry worldwide. PRV is the cause of Aujeszky's disease, also known as pseudorabies. Clinical signs are related to respiratory and nervous systems, which are the preferred site for PRV replication. Also, PRV infection can cause a high mortality in neonatal piglets, abortion in sows, and loss of body condition in growing pigs. The feral swine population is known to be infected with PRV strains, and considered to be a threat for the commercial swine industry. Real-time polymerase chain reaction (real-time PCR) is a valuable diagnostic technique that can rapidly identify nucleic acid of infectious agents in clinical specimens.
Methods: A real-time PCR assay based on the gB and gE genes was designed to identify PRV nucleic acid in diagnostic samples. Using virus isolation as the gold standard test, the assay performed well in a variety of diagnostic matrices.
Results: Initial testing was conducted on 711 nasal swabs (sensitivity: 97.1% [95% confidence interval (confidence interval, CI): 95.8-98.8%], specificity: 78.6% [95% CI: 71.2-86.1%]); 258 brain tissues (sensitivity: 100.0% [95% CI: 100.0%], specificity: 92.1% [95% CI: 88.7-95.5%]); 132 bronchial-alveolar lung fluid specimens (sensitivity: 100.0% [95% CI: 100%], specificity: 76.9% [95% CI: 69.3-84.6%]); 29 preputial swabs fluid samples (sensitivity: 100.0% [95% CI: 100%], specificity: 88.5.5% [95% CI: 76.2-100%]); and 144 tonsil samples (sensitivity: 52.5% [95% CI: 37.0-68.0%], specificity: 93.3% [95% CI: 88.5-98.1%]). Low numbers of other sample matrices showed good agreement between results of virus isolation and PCR.
Conclusions: Diagnostic performance of the real-time PCR assay developed as a testing method indicates that it is a rapid, accurate assay that is adaptable to a variety of PCR platforms and can provide reliable results on an array of clinical samples.

063P

The porcine antibody repertoire and its response to PRRSV infection.

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Antibody responses are critical to effective immunity to viral infections. Thus, extensive efforts have been directed to characterize the antibody and neutralizing antibody responses to PRRSV infection, in the hope of elucidating key insights into protective and cross-protective immunity. Despite these efforts, the role of antibody responses in PRRSV immune protection remains poorly understood. To address this, we characterized the expressed immunoglobulin repertoires in healthy and JA142 PRRSV-infected pigs using amplicon-based 454 high-throughput sequencing. Bioinformatic analysis of approximately 450 thousand reads revealed preferential usage of CDR3s of specific lengths in the infected pool. These PRRSV-specific CDR3 lengths corresponded with unique sequences that accounted for between 11 and 35 percent of all transcripts in their respective CDR3 size class. Furthermore, these same sequences were rare (~0.1%) in the uninfected pool. Diversity analysis estimated the size of the porcine heavy chain immunoglobulin repertoire to be approximately 3.5x10^5, an estimate similar to that reported in humans, suggesting that the swine antigen-binding repertoire is similarly complex, despite the apparent lack of diversity in the porcine heavy chain variable gene (IGHV) framework regions. As a consequence of their repertoires being dominated by a small number of sequences, pigs infected with PRRSV showed a decrease in their total repertoire diversity. Furthermore, PRRSV-specific IGHV gene segment usage was dominated by IGHV4/IGHV10, suggesting a possible immunogenetic component of PRRSV immunity. We expect the results of this research to open the door to development of therapeutic reagents to treat acute PRRS, genetic testing for PRRS resistance, and a mechanistic understanding of cross-protective immunity.

064P

Toll-like receptors and pro-inflammatory cytokine expression profile of porcine intestinal epithelial cells upon stimulation with enterotoxigenic *Escherichia coli*.

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Enterotoxigenic *Escherichia coli* (ETEC) infection is of global importance because of its high morbidity and mortality rate in humans and animals. ETEC has been the principal agent causing the traveler's diarrhea in humans and post-weaning diarrhea in pigs. Intestinal innate immunity is the first line of defense in enteric bacterial infections. The pathogenicity of ETEC is attributed to the fimbriae and heat labile (LT) and heat stable (ST) toxins. Porcine intestinal epithelial cells (IPEC-J2) provide suitable in vitro model for studying ETEC infection pathogenesis as shown in our previous studies. This study was conducted to analyze the changes in the gene expression of toll-like receptors (TLRs), pro-inflammatory cytokines and chemokines upon stimulation of IPEC-J2 cells with different strains of ETEC for 3 and 6h. We used wild type ETEC strains with K88ac fimbriae 3030-2 (O157:K87 LT+, STb+), 2534-

064P (continued)
86 (O8:K87: NM: LT-I+, STb+), 1836-2 (LT$^-$, ST-, astA+), and G58-1 a non pathogenic, non-fimbriated, wild-type (O101:K28: NM, LT$^-$) at multiplicity of infection 10:1. G58-1 and cells with media alone served as negative controls. Cell lysates were processed for total RNA extraction and total RNA was used to prepare cDNA using reverse transcriptase reagents. The gene expressions for TLRs and cytokines were quantified using real time RT-PCR. We found that wild type strains 3030-2 and 2534-86 down regulated TLR-7 and up regulated pro-inflammatory cytokines, IL-1 α and TNF-α at both 3 and 6h compared to the negative control. Both these wild type ETEC strains also significantly increased the CCL-20 expression. Non-fimbriated strain G58-1 did not induce any change in the gene expressions showing that fimbrial attachment is a prerequisite for the pathogenesis and production of pro-inflammatory cytokines. Further investigations will help in understanding more about enteric mucosal innate immune responses to ETEC which may potentially contribute to its prevention and treatment.

065P
Modulation of antimicrobial Host defense peptide gene expression by free fatty acids.
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Widespread use of antibiotics in food animal production for growth promotion and disease prevention is suspected to be a major driving force for rapid emergence of antimicrobial resistant pathogens. Antibiotic alternative approaches to disease control and prevention are urgently needed. We showed previously that butyrate, a major species of short-chain fatty acids (SCFAs) fermented from undigested fiber by intestinal microflora, is a potent inducer of endogenous antimicrobial host defense peptide (HDP) genes and that dietary supplementation of butyrate reduces the titer of Salmonella enteritidis in the chicken cecum following experimental infections. Here we extended our earlier studies by testing the capacity of free fatty acids of different carbon chain lengths to induce the HDP gene expression in chicken HD11 macrophages and primary monocytes. Real-time RT-PCR revealed that HDP gene expression is inversely correlated with aliphatic carbon chain length of free fatty acids, with SCFAs being the most potent, medium-chain fatty acids moderate and long-chain fatty acids largely ineffective in stimulating HDP synthesis. In addition, three SCFAs, namely acetate, propionate, and butyrate, exhibited a strong synergy in augmenting HDP synthesis in chicken cells. Consistently, supplementation of chickens with a combination of three SCFAs resulted in a further reduction of S. enteritidis in the cecum as compared to feeding of individual SCFAs. Importantly, we found that free fatty acids enhance HDP gene expression without provoking interleukin 1β production. Taken together, fatty acids and SCFAs in particular are capable of boosting innate immunity and disease resistance with a minimum impact on the proinflammatory response. Oral supplementation of SCFAs may have potential for further development as a promising antibiotic alternative approach to disease control and prevention in animal agriculture.

066P
Mucosal immunity in chickens infected with low-pathogenic avian influenza viruses.
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It was studied the humoral and cellular immune response in chickens 28-40 day-old after infection with low-pathogenic avian influenza viruses H5 and H13 isolated from wild waterfowl. Intranasal and intravenous infection at different doses no clinical signs for 21 days. The virus was not reisolated from internal organs (trachea, lungs, spleen, intestines), but the gene was found in internal organs at 3, 7, 10, 14, 21 days after infection. Specific antibodies were found in 20-100% of poultry on 6-10 days after infection. Infection was caused suppression of immune reactions in the cell subpopulation CD4, CD8, IgM, IgG, IgA, and macrophages in the first 5 days, since 7th day it was observed a sharp increase in activity of immune cells of internal organs (lungs, trachea and spleen), which lasted until the end experiment.

067P
Genome-wide histone methylation analysis in MD-resistant and MD-susceptible chickens after MDV infection.
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Purpose: Histone modifications are important epigenetic features related to the activation and repression of genes. Although virus-induced histone modifications were observed for single genes, there is still a lack of evidence for the global histone modifications induced by viruses. Here, we used the chicken Marek's disease model to identify genome-wide H3K4me3 and H3K27me3 alterations induced by Marek's disease virus (MDV) infection.
Methods: The chromatin immunoprecipitation combined with massively parallel sequencing (ChIP-Seq) method was used to detect the H3K4me3 and H3K27me3 enrichment in two chicken lines either resistant or susceptible to Marek's disease (MD) in a Marek's disease virus (MDV) challenge trial.
Results: A modest H3K4me3 and H3K27me3 enrichment within the chicken genome was observed relative to human and other species. While H3K4me3 is positively associated with gene expression level, H3K27me3 is negatively associated with gene expression level in transcription start sites (TSS) and genic regions. It was found that MDV infection caused large amounts of histone modifications within the genome. However, not all of the histone modifications altered gene expression alteration. By separating the genes into different functions, such as the genes involved in antibacterial function, there were stronger correlations between histone modification and gene expression, particularly in several candidate genes such as CD8α, IL8 and CTLA4.
Conclusion: The histone modification profile induced by MDV infection gives us clues of candidate genes that may involved in MD-resistance and -susceptibility.

068P
Decreased permeability changes in bovine epithelial cells treated with P2X7 receptor antagonists prior to exposure to adenosine triphosphate.
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Purpose: *Mannheimia haemolytica* infections in calves are associated with extensive leakage of blood products into the air spaces of the lung. One possible mediator of this leakage is adenosine triphosphate (ATP). Experiments with bovine lung endothelial and epithelial cells demonstrate a significant increase in their permeability when exposed to 1mM ATP. This effect is likely mediated by interaction of ATP with the $P2X_7$ receptor, but other purinergic receptors may play a role in this process as well. In the present study, several $P2X_7$ receptor antagonists, periodate-oxidized ATP (oATP), Coomassie brilliant blue G (BBG), KN62, and A438079, were compared to determine whether blocking this receptor would prevent the permeability change associated with ATP exposure. In addition, the specificity of the individual antagonists for the $P2X_7$ receptor was evaluated.
Methods: We used Mac-T cells grown on tissue culture inserts to perform trans-epithelial electrical resistance (TEER) as a measure of epithelial monolayer

068P (continued)
permeability. For the $P2X_7$ receptor antagonist specificity experiments, calcium influx into the epithelial cells was determined using a plate reader to measure the fluorescence of epithelial cells loaded with the Fluo-3 acetoxymethyl ester.
Results: All of the receptor antagonists used in this study were able to decrease the permeability changes in bovine epithelial cells associated with ATP exposure.
Conclusions: It appears extracellular ATP interactions with the $P2X_7$ receptor on epithelial cells is associated with permeability changes in those cells.

069P
Deciphering the impact of maternal cells in neonatal health and immune development.
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Purpose: Mortality and decreased weight gain resulting from infection and disease in dairy calves is a persistent problem within the dairy industry, increasing costs of production. The bovine neonate relies solely on colostrum quality and timing of feeding for the acquisition of antibodies through passive transfer. To date, colostrum quality is determined by concentration of antibodies. However, colostrum also contains proteins and cells which may enhance immune development in the neonate. Colostral cells cannot survive flash freezing, therefore feeding of frozen colostrum was compared with fresh colostrum to determine transfer of immune cells on calf health. The aim of this study was to analyze the impact of non-antibody colostral components on long-term immune development.
Methods: Thirty-seven female Holstein and Jersey calves were bottle-fed (or esophageal fed) 4 quarts total of either fresh or frozen colostrum at birth. Colostrum was analyzed for anti-bacterial milk proteins by ELISA and mastitis pathogens by standard biochemical tests. Calf peripheral blood samples were taken before and after feeding colostrum as well as on day 1, 3, 7, 14, 21, 28, and subsequently once a month. Blood and colostrum were analyzed for antibody and cytokine levels by ELISA and cell profiles were determined by flow cytometry.
Results: Treatment showed no significant difference between fecal scores or respiratory condition, however, on days 37-41 calves fed frozen colostrum tended to have higher respiratory scores.
Conclusions: We hypothesize that differences in immune development will be observed in older animals depending on treatment. The findings of this study will provide novel information on the impact of maternal immune cell transfer through colostrum in immune development in offspring.

070P
Proteomic evaluation of protein modulation in bovine lung tissue following *in vivo* challenge with Mannheimia haemolytica pneumonia.
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Bovine respiratory disease (BRD) requires widespread use of antibiotics for treatment and prevention, which has led to food safety concerns regarding the potential for drug residues in edible tissues as well as an increased selective pressure for the emergence of resistant strains of bacteria. Accordingly, there exists a need to develop veterinary therapeutics with broad spectrum activity, minimal side effects, and to which bacteria will not easily develop resistance. Modulation of proteins in healthy versus diseased bovine lung tissue was examined to identify target candidates for alternative therapies or diagnostic biomarkers. Antimicrobial peptides (AMPs) are attractive candidates for use as novel veterinary therapies primarily because AMPs exhibit specificity, broad-spectrum activity, and it is deemed unlikely bacterial pathogens could easily develop resistance to AMPs. To expand current knowledge of AMP expression during acute bovine lung disease, protein changes were profiled in lung tissue samples collected from control, sham-infected, and steers genuinely infected with Mannheimia haemolytica. Proteins were extracted, in-solution digested, and analyzed using nanoflow liquid chromatography coupled with tandem mass spectrometry (nano LC-MS/MS). Preliminary data indicated that proteins identified in diseased lung tissues when compared to control tissues could be broadly categorized as vascular derived, acute phase, complement factors, cell motility, antimicrobial, and proteins involved in oxidation-reduction. Analysis of LC-MS/MS normalized peptide counts from in-solution digests allowed for the measure of relative protein abundance. In diseased bovine lung tissue, the AMPs cathelicidin-1, and -7, as well as complement C3, haptoglobin, and inter-α-trypsin inhibitor heavy chain H4 showed increased relative peptide abundance when compared to healthy lung tissue. The results aided in the further characterization of the expression of proteins related to the host response to BRD, specifically AMPs, which could aid in the identification of targets for therapeutic development and biomarkers for evaluating efficacy of new and existing drugs.

071P
Expression of CXCR1 and CXCR2 in bovine mammary tissue.
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Mastitis is an ongoing issue in the dairy industry. Our lab has previously determined an association between mastitis infections and polymorphisms in the interleukin-8 receptor, CXCR1. However, in bovine mammary tissue, little is known about which cell populations express, and to what extent those cell populations express CXCR1 and its sister receptor CXCR2. Purpose: We hypothesize that CXCR1 and CXCR2 will be expressed in mammary tissue based on the expression of both receptors in human mammary stem cells and breast cancer cells. Methods: To this end, our study is in the process of evaluating mammary tissue sections from dairy cows using dual immunofluorescence. Results: Preliminary results from 4 Holstein dairy cows show an abundance of both CXCR1 and CXCR2 expression in both ductal and alveolar epithelial cells and little to no expression in connective tissue. Of interest, there appears to be areas of intense epithelial staining, but the basis for this is not currently clear. Conclusion: These preliminary results suggest that bovine mammary epithelial cells express both CXCR1 and CXCR2. Activation of these receptors on mammary epithelial cells is highly probable during infection because the ligand for these receptors, IL-8, is released by mammary epithelial and surrounding cells following exposure to bacteria or other stressful conditions. Deducing the quantitative and qualitative expression of CXCR1 and CXCR2 in the mammary tissue, as well as the functional response could provide a potential avenue for prevention and control of mastitis.

072P
Strain virulence affects *Mycobacterium bovis*-infected bovine macrophage gene expression.
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072P (continued)
Bovine tuberculosis caused by *Mycobacterium bovis* (*M. bovis*) leads to economic losses in animal production and has an impact on public health. Macrophages play an essential role in the immune response against *M. bovis*. Analysis of macrophage gene expression may reveal the molecular mechanisms associated with pathogenesis of bovine tuberculosis. Purpose: In this study, we aimed to identify macrophage early gene expression after *M. bovis* infection. Methods: In order to accomplish our objective, we infected bovine macrophages with *M. bovis* virulent (AN5) and non virulent (BCG) strains, using a MOI of 10:1 during 4 hours. RNA was extracted for gene expression analysis on affymetrix microarrays and real time PCR. Results: Gene expression had a higher intensity and was 5 times higher in RNA of macrophages infected with the avirulent strain BCG. mRNA transcripts induced by BCG mainly include genes associated to oxidative and apoptosis processes, suggesting that gene expression is oriented to bacteria removal. On the other hand, AN5 induced transcription of genes involved in metabolic pathways mainly and a small number of immune response related genes, suggesting that the virulent strain directs macrophage transcription to generate a niche that allows bacteria survival and dissemination. Conclusion: The strain virulence plays a significant role that affects macrophage gene expression. This work was supported by project: CONACYT-CB-2005-24794

073P
The role of regulatory T cells in immune responses to *Mycobacterium avium* subspecies *paratuberculosis* (Johne's disease) in cattle.
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Johne's disease is a chronic gastrointestinal disease of ruminants caused by infection with *Mycobacterium avium* ss. *paratuberculosis* (MAP). In addition to its mounting financial burden, Johne's disease may have zoonotic potential and has been targeted as the cause of some cases of human Crohn's disease. Previously, we proposed that regulatory T cells (Tregs) (CD4+/CD25+/FoxP3+) may have an inhibitory effect on the vital proinflammatory Th1 immune response to MAP. Inhibition of Th1 responses would allow MAP to infect more cells and avoid eradication by the host immune system. To study this, blood samples taken from both infected (n=27) and control (n=12) cows were used to generate peripheral blood mononuclear cells (PBMCs). PBMCs were cultured in media for 72 hours with or without stimulation by whole MAP. Flow cytometric results show significant up-regulation of activated T cell populations (CD4+/CD25+) in PBMCs from infected cows, relative to PBMCs from control cows following stimulation with MAP (7.31% vs. 3.48%, p-value < 0.05), as expected. The infected group also had a significantly greater relative percentage of Treg cells (CD4+/CD25+/FoxP3+) in lymphocyte populations than the control group, regardless of stimulation (p-value < 0.05). Concurrent immunohistochemical studies in our laboratory suggested that the highly-prevalent bovine leukemia virus (BLV) may be affecting the immune system of our study cows. Preliminary results indeed show that Treg populations in PBMCs from BLV positive cows are reduced upon MAP stimulation, while PBMCs from BLV negative cows show an up-regulation of this cell population (p-value < 0.1). Immunohistochemical staining of tissues supports the notion that BLV has a negative impact on the relative percentage of Tregs present. BLV may thus reduce overall Treg numbers and limit their response to stimulation with MAP antigens. One intriguing and very testable consequence of this would be a higher percentage of BLV positive cows developing clinical Johne's disease due to unchecked inflammation. This possibility is currently being investigated as is the possible functional consequence of MAP antigen-specific Tregs in the absence of BLV.

074P
Salmonella serovars differentially stimulate bovine leukocyte responses *in vitro*.
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The majority of *Salmonella* serovars cause no clinical signs in cattle, while some serovars, such as *Salmonella enterica* serovar Typhimurium (ST) and Dublin (SD), may cause severe disease. Mechanisms underlying the differences in pathogenesis between different serovars are not clear. The objective of this study was to determine innate immune responses of bovine leukocytes exposed *in vitro* to SD, ST, and *Salmonella enterica* serovar Enteritidis (SE), and their role in development of pathogenesis and host specificity. Jugular blood was collected from 10 Holstein calves at approximately 3 weeks of age and infected *in vitro* with non-opsonized or serum-opsonized SD, ST, and SE for 2 hours. Leukocyte phagocytosis and oxidative burst, cell surface expression of cluster of differentiation (CD) 14 and CD18 were analyzed using flow cytometry. Leukocyte mRNA expression of interleukin-8 (IL-8), IL-12, tumor necrosis factor (TNF)-α, and toll-like receptor 4 (TLR4) were determined using qRT-PCR. Results indicated that SD exposure, not ST or SE exposure, increased cell surface CD14 ($P = 0.006$). Opsonized SD failed to increase cell surface CD14 ($P > 0.05$). Only SE exposure increased oxidative burst ($P = 0.025$). In addition, opsonized SD and ST increased oxidative burst in blood leukocytes ($P < 0.001$ and $P = 0.002$, respectively). All 3 serovars increased cell surface CD18 and mRNA expression of TNF-α, IL-8, and IL-12. However, none of these serovars affected mRNA expression of TLR4. Our results suggested that, compared to ST and SE, bovine-host-adapted SD enhanced its uptake by bovine phagocytic immune cells by upregulating CD14 expression on host cell surfaces. Additionally, SD as well as ST, suppressed the oxidative burst in phagocytes, which benefits their survival, replication, and dissemination within host cells. In contrast, SE did not induce uptake by phagocytes and showed no oxidative burst suppressing effect. Thus, SE exposure in cattle is likely to be constrained to the gastrointestinal tract, and eliminated at the early stage of infection. Results also indicated that antibodies in serum control SD infection through downregulation of CD14 and upregulation of oxidative burst.

075P
Identification of immunorelevant (IR) genes in Brucella abortus infected bovine dendritic cells by suppression subtractive hybridization.
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Background: Brucella abortus is a gram-negative facultative intracellular pathogen of cattle, and an important zoonosis in humans worldwide. Brucella requires an IFNγ and IL-12 mediated T helper type 1 (TH1) response for bacterial clearance, and persistent infections in humans and immunodeficient mice have been associated with a T helper type 2 (TH2) response. The role of DC in initiating the appropriate adaptive immune response makes this cell type pivotal in the course of Brucella infection. Previous studies have shown that dendritic cells (DC) from humans and mice are highly permissive for Brucella survival and proliferation.
Purpose: To identify novel genes that are differentially expressed with infection to provide targets for future studies of Brucella pathogenesis.
Methods: Monocyte- derived DC (mdDC) were cultured from bovine peripheral blood mononuclear cells (PBMC) using the recombinant bovine cytokines IL-4 and GM-CSF. The resulting mdDC were DEC205+, MHC class IIhi. MdDC were infected with B. abortus strain 2308 at an MOI of 1. Suppression subtractive hybridization was used to identify differentially expressed genes in infected and control mdDC cultures.
Results: A total of over 200 clones were obtained, sequencing revealed 49 unique sequences. Based on current literature, 24 of these genes were selected as immunorelevant (IR) and differential expression of these genes will be validated using RT-PCR on similarly infected bovine mdDC. Several genes involved in apoptosis and regulation of apoptosis were identified, which could be involved in mediating Brucella spp. documented ability to inhibit

075P (continued)
apoptosis in infected cells. Genes with functions relating to antigen processing and presentation, signal transduction, vesicular transport and immune response in general may have interesting implications for the mechanism behind the stealthy strategy of Brucella and its avoidance of eliciting an immune response and alter its endosomal membrane and traffic towards the endoplasmic reticulum.
Conclusion: Suppression subtractive hybridization is a useful technique for identifying novel genes to direct further research into the pathogenesis of B. abortus.

076P
Identification and characterization of molecules suitable for improved diagnostics for Contagious Bovine Pleuropneumonia (CBPP).
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Background: Contagious bovine pleuropneumonia (CBPP) is considered by the AU-IBAR to be one of the most important livestock diseases in sub-Saharan Africa. The current diagnostic assays have limited sensitivity.
The aim of the project is to comprehensive characterization immune responses against Mycoplasma proteins in infection and the subsequent identification of potential proteins for incorporation into improved diagnostic assays for Contagious Bovine Pleuropneumonia (CBPP), which is caused by *Mycoplasma mycoides subspecies mycoides* (*Mmm*).
Objectives:
1.Screening of 80 recombinant antigens using sera from experimentally infected cattle
2.Characterize humoral immune responses in different stages of disease
Methods: We expressed 24 recombinant Mycoplasma Mycoides subspecies mycoides (Mmm) proteins. Of these, 83% (20/24) were recognized by Mmm experimentally infected cattle sera.
These 20 recombinant proteins were further analyzed in sera from a vaccine trial and CBPP outbreaks in Kenya.
Results: Five out of twenty recombinant proteins were significantly recognised in experimental sera and highly recognised in the vaccination and outbreak sera.
Conclusion: Our study illustrates the potential of this approach to identify candidate diagnostic antigens which also may be related to protective immune responses and justifies further investigation.

077P
How long are viruses detected by PCR in blood and nasal swabs of calves vaccinated with infectious vaccines?
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PCR is a very sensitive technique to aid in the diagnosis of clinical disease in cattle. Currently, many diagnostic laboratories use this technique to detect bovine viral diarrhea virus (BVDV), bovine respiratory syncytial virus (BRSV), bovine herpesvirus (BHV-1), parainfluenza virus (PI-3), and other viruses of cattle such as bovine coronavirus (BoCV). Although viral nucleic acid detected by PCR does not always correlate with presence of infectious virus, a positive PCR result is often presumed to indicate recent or current infection. The present study was designed to determine the period of time that various samples (e.g. blood, nasal swabs) are positive by PCR after vaccination of beef and dairy calves with infectious (modified live) vaccines. Results of this study show that specific viruses can be present at various times up to 3 weeks post-vaccination, depending on the virus, the vaccine, and the animal. These results need to be considered when using PCR to make a diagnosis of clinical disease in recently vaccinated cattle. Revaccinated adult cattle can also shed virus, but they are less likely to be PCR positive, and then only for a single virus and only for a short period of time because they are often already immune and the vaccine virus does not infect and replicate in the animal.

078P
Production of mycobacterial recombinant proteins ESAT-6 and CFP-10 for bovine tuberculosis diagnosis.
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Purpose:In recent years, there has been emphasis on the development of new antigens for the diagnosis of tuberculosis. Many antigens have been proved in serological and cell-mediate immune tests. After all, ESAT-6 and CFP-10 have shown a strong potential because they are recognized in TB infected cattle, and they do not elicit a response in TB free cattle. Therefore, its production in pure and large quantities is essential for the development of cost-effective test kits. Unfortunately, mycobacterial protein expression in heterologous host has proved to be difficult and in most cases the proteins were expressed in the insoluble form that could only be purified and refolded in low yields after time consuming and labor intensive process. The later produce differences in the folding of the protein that could induce different immunological responses. The aim of the current study was to express and purify the ESAT-6 and CFP-10 proteins.
Methods: Overnight cultures of *E. coli* strains C41 and M15 harboring recombinant plasmid expressing the ESAT-6 and CFP-10 proteins, respectively were grown at 37°C in LB broth with vigorous shaking to an OD_{600} nm of 0.6 after which recombinant protein expression was induced by 250μM IPTG for another 4 hours. The ESAT-6 protein was found as inclusion bodies, these were dissolved and metal affinity purified under denaturing conditions, then it was refolded by dialysis with decreasing urea concentrations. The CFP-10 protein was expressed in soluble form and this was cationic-exchange purified. All the fractions collected were analyzed by SDS-gels and Western blot with a monoclonal anti-His and polyclonal anti-CFP-10 antibodies.
Results: Both proteins have N-terminal six-His tag in order to facilitate its purification and identification, but only the ESAT-6 protein was identified by the anti-his antibody and metal affinity purified. While the CFP-10 protein was not recognized by the anti-his antibody and neither purified by metal affinity.
Conclusions: These suggest differences in the folding of the protein that could induce diverse results in the common diagnostic test for bovine tuberculosis.
This work was supported by project: PAPIIT IN-214009-3.

079P
The effect of vitamin E supplementation on an experimental Haemonchus Contortus infection in lambs.
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Gastrointestinal parasitism in sheep, particularly lambs, results in substantial economic losses to producers worldwide. Nutritional status has been shown to play an important role in host immune response to parasitic infections. Purpose: The objective of this study was to investigate the effect of vitamin E

079P (continued)
supplementation on an artificial Haemonchus contortus (H. contortus) infection in immature lambs.
Methods: Twenty Dorset lambs were stratified into two treatment groups according to parasite susceptibility. Worm-free lambs, 28 to 32 weeks of age, were supplemented with vitamin E (d-α-tocopherol) for twelve weeks following the recommendations of the National Research Council for minimal daily requirement (VE5; 5.3 IU/kg body weight (BW)/day (d), n=10) or optimal immune function (VE10; 10 IU/kg BW/d, n = 10). Five weeks after initiation of vitamin E supplementation, lambs were infected with 10,000 H. contortus L3 larvae. Samples were taken weekly for determination of fecal egg counts (FEC), packed cell volume (PCV), serum α-tocopherol and immunoglobulins (I; IgG, IgE). After six weeks of infection, blood was collected to determine Th1 (IFN- γ) and Th2 (IL-4) cytokine expression and the lambs were humanely slaughtered for the determination of tissue vitamin E content, worm burden and histologic evaluation of the abomasum.
Results: Increased dietary vitamin E supplementation had no effect on liver (P=0.08), muscle (P=0.62), or lymph node (P=0.38) vitamin E content. There was no effect of treatment on FEC or PCV, however there was a 49% reduction in total abomasal worm burden (P=0.002) in the VE10 group. There was a three-fold increase in relative gene expression for IFN-γ in VE10 but no effect on IL-4. Pending analyses will examine clinical pathology of the abomasum and serum α- tocopherol and immunoglobulin concentration.
Conclusions: Elevated levels of vitamin E supplementation had a beneficial effect on the abomasal worm burden and increased expression of Th1 cytokine IFN- γ however, there was no treatment effect on PCV or FEC.

080P
The effect of age on telomerase activity and reactive oxygen species (ROS) production in plasma and peripheral blood mononuclear cells of horses.
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The Gluck Equine Research Center, Lexington, KY, USA.

Cellular senescence is a hallmark characteristic of the aging immune system. We have previously shown that telomeres shorten with increasing age in the horse, a mechanism that limits proliferation of immune cells. Telomere shortening is counteracted by the cellular reverse transcriptase enzyme, telomerase. Moreover, oxidative stress is thought to increase with age and pose a threat to the telomere maintenance system, in particular telomerase activity. Purpose: We hypothesize that old horses when compared to young will have decreased telomerase activity correlated with increased ROS production, decreased telomere length and proliferative responses. Methods: Peripheral blood samples were collected from ten young (1-2 yrs) and ten old (20-30 yrs) horses of mixed breed and sex. Telomerase activity was measured using the telomeric repeat amplification PCR (TRAP) method, which determines the ability of telomerase to synthesize telomeric repeats onto an oligonucleotide substrate. A control 293T tumor cells line was used as a positive control, and a TSR oligonucleotide with a sequence similar to telomere primers was used to generate a standard curve for quantitation of telomerase activity in freshly isolated and PHA-stimulated (24 hrs) peripheral blood mononuclear cells (PBMC). In addition, PBMC proliferation was measured by thymidine incorporation and telomere length was determined by using the telomere FLOW-FISH method. Reactive oxygen species (ROS) production in both plasma samples and PBMC was determined using the TBARs assay (Thiobarbituric Acid reactive substances), which measures lipid peroxidation, a major indicator of oxidative stress. Results: Aged horses when compared to young had significantly ($P<0.05$) reduced telomerase activity from stimulated PBMC which correlated with decreased proliferative responses and decreased telomere length. Further, aged horses had significantly ($P<0.05$) increased levels of ROS in plasma samples and in freshly isolated PBMC. Conclusions: ROS production potentially contributes to decreased telomerase activity which plays a role in immunosenescence of the aged horse.

081P
Antibody and antigen-specific intradermal response of foals and adult horses vaccinated with a live attenuated vaccine.
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Purpose: Equine neonates have decreased humoral and cell-mediated immune responses compared to adult horses after administration of killed vaccines. However, young foals mount strong humoral and cell-mediated immune responses after experimental infection with Rhodococcus equi. As a basis for this study, we hypothesized that newborn foals can mount strong immune responses after vaccination with live Mycobacterium bovis BCG. The objective of this study was to compare vaccine-specific serum immunoglobulin concentrations and delayed type hypersensitivity (DTH) reactions between newborn foals (n=7), 4-month old foals (n=7), and adult horses (n=6) after a single subcutaneous immunization.
Methods:Relative immunoglobulin concentrations were measured by ELISA prior to, and at 2, 4, 6, and 8 weeks after vaccination. Eight weeks after vaccination, DTH was assessed by measuring the increase in double skin thickness after intradermal injection of purified protein derivative.
Results:Both groups of foals and adult horses responded with a significant increase in vaccine-specific total IgG, IgGa, IgGc, IgG(T), and IgM concentrations. In contrast, only adult horses mounted a significant IgGb response. Vaccine-specific concentrations of IgGa were significantly different between all groups, with the highest concentrations in adult horses followed by 4-month-old foals and finally newborn foals. Total IgG and IgM concentrations were significantly higher in adult horses than in either group of foals. Immunized horses had significantly higher DTH responses than age-matched controls. DTH responses were significantly greater in both groups of foals than in adult horses.
Conclusions:Foals have lower immunoglobulin production but stronger DTH responses than adult horses after immunization with BCG.

082P
Comparison of the ability of two different adjuvants to stimulate antigen presenting cells function *in vivo*.
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Purpose: Adjuvants are included with many inactivated and some modified live vaccines to enhance immune responses to specific antigens. While early vaccines relied exclusively upon aluminum salts, still the major adjuvant used in human vaccine, other adjuvant products are used in veterinary medicine. In addition to enhancing antigen presentation, adjuvants can also enhance the development of specific immune responses. Thus, alum adjuvants often preferentially stimulate humoral immune responses. By contrast, lipid-based adjuvants are often more effective at stimulating cell-mediated immune responses. Metastim® is a lipid-based adjuvant which is reported to elicit both serological and cellular immune responses, though the mechanism responsible for its activity remains unclear. In this study, we compared the ability of two equine influenza virus vaccines containing either Metastim® or alum to stimulate antigen presenting cell function in vivo.
Methods: Six ponies were intradermally inoculated with inactivated equine influenza (KY97) and either adjuvant with saline included as a negative control. Multiple sites were injected so that biopsies could be collected at different times post injection. The 4mm punch biopsies were formalin-fixed, paraffin-embedded, and stained with hematoxylin and eosin (H&E). Total RNA was isolated from 2mm punch biopsies for the determination of gene expression by real-time PCR.
Results: H&E staining revealed a variety of cells recruited to the injection sites, including lymphocytes, neutrophils, eosinophiles and macrophages. Both

082P (continued)
vaccines stimulated gene expressions of IL-1, TNFα, MCP-1, MHC II, CD86, IL-5 and thymic stromal lymphopoietin (TSLP). The vaccine containing Metastim® elicited significantly higher gene expression of IL-10, IL-12, interferon-γ, TNF-α and CD163 compared to alum ($p<0.05$).
Conclusions: While both adjuvants stimulated the production of cytokines and accessory molecules at the injection site, the significant induction of IL-12 and IFNγ indicates that Metastim® can elicit Th-1 immune responses more effectively than the aluminum salt.

083P
Neutralizing antibody-mediated control of equine infectious anemia virus infection in the absence of T lymphocytes.
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Purpose: Vaccines that prevent HIV-1 infection will likely elicit antibodies that neutralize diverse strains. However, the capacity for lentiviruses to escape broadly neutralizing (BN) antibodies while retaining replication fitness is not completely understood, nor is it known whether neutralizing antibodies alone can control heterologous infection. Equine infectious anemia virus (EIAV) is a lentivirus that causes persistent infection in horses. EIAV-infected horses are able to mount BN antibody responses that reduce levels of replicating virus during long-term inapparent infection. The purpose of this study was to determine if immune plasma and purified immunoglobulin (Ig) from horses persistently infected with EIAV would provide protection against viral challenge in the absence of T lymphocytes.
Methods: We infused immune plasma and purified Ig from 2 horses persistently infected with EIAV that was able to neutralize homologous virus and several envelope variants containing heterologous surface units (SU) into foals affected with SCID. This was followed by challenge with a homologous EIAV stock that contained a distinct SU variant (designated $WSU5_{\Delta V3}$) at low copy number. Single genome amplification was used to sequence virus from foal plasma.
Results: Four SCID foals treated with immune plasma from one persistently infected horse (A2150) were protected against clinical disease, with prevention of infection occurring in one. Interestingly, selection of $WSU5_{\Delta V3}$ occurred in 3 SCID foals. Infusion of immune plasma from a second persistently infected horse (A2140) into a SCID foal prior to EIAV challenge resulted in complete protection. Infusion of purified Ig from this plasma into 4 additional SCID foals prior to EIAV challenge resulted in viremia in one foal, which was associated with $WSU5_{\Delta V3}$. However, when an increased dose of purified Ig was used, all remaining foals were protected against infection, indicating that the resistant $WSU5_{\Delta V3}$ variant was effectively neutralized.
Conclusions: In the complete absence of T cells, BN Ig can prevent lentivirus infection and clinical disease. This work may have implications for prevention of lentiviral disease, including HIV-1.

084P
How long do memory cells persist in the dog vaccinated with Leptospira canicola, icterohaemorraghiae, grippotyphosa, and pomona?
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Dogs aged 12 weeks or older must receive two or more doses of leptospira bacterin 2 to 6 weeks apart during the primary vaccination program in order to develop an immune response. After the initial priming and immunizing doses, it is recommended that revaccination occur on a yearly basis with a single dose of vaccine. It is not known how long immunologic memory persists after the initial 2 doses, which is required for a single subsequent dose of vaccine to boost the response. When immunologic memory is absent, a single booster dose will fail to stimulate a response. Since it is common for many dogs to lose their ability to continue to produce antibody to one or more of the 4 serovars of leptospira six or more months after initial vaccination, it is not certain how long immunologic memory persists. To answer this question, dogs were initially vaccinated 2 times at either 12 or 13 weeks and again at 15 or 17 weeks of age. They were then not revaccinated until 3or 4 years later to determine if the dogs previously vaccinated developed antibody after a single dose of vaccine. Immunologic memory cells must persist to get a response with one dose of vaccine. In contrast, if memory does not persist, two doses of vaccine 2 to 6 weeks apart will be required to get an antibody response, as is required the first time the dog is vaccinated with this bacterin when it has no memory cells. The results of this study suggest that dogs not revaccinated for several years (>3 years) should receive 2 doses of vaccines 2 to 6 weeks apart. These results also suggest memory cells do not persist, as the response was similar in dogs vaccinated more than 3 years earlier as it was to the initial vaccination program when the dog was naive. Immunologic memory for IgG antibody is short lived to leptospira bacterins. In contrast, in previous studies we have found via skin testing that IgE memory responses persist 4 or more years.

085P
Are recently vaccinated puppies protected from challenge with multiple isolates of canine distemper virus (CDV)?
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University of Wisconsin-Madison, Madison, WI, USA.

It has been suggested that current canine distemper virus (CDV) vaccines do not provide protection from all variants of CDV in the US. This assertion stemmed from observations of CDV outbreaks in puppies and adult dogs in animal shelters and a few pet stores only. To date, no studies have been done to demonstrate significant antigenic differences among the genotypic variants of CDV, nor have any challenge studies been reported. To determine the validity of this claim, we performed a challenge study using 6 twelve-week old dogs which had recently been vaccinated with Duramnune® Max5 (Boerhinger-Ingleheim Animal Health, St. Louis MO) and a group of similar aged, unvaccinated control dogs. Five weeks after vaccination, all pups were challenged intravenously with 12 different isolates of CDV. These isolates were collected from CDV outbreaks around the US, including some from dogs that had been vaccinated, but developed CDV disease and from raccoons. After challenge, dogs were monitored daily for clinical signs, and blood, nasal and rectal swabs were collected to detect challenge virus shed via PCR assay. Of the dogs which had not been vaccinated, all developed severe distemper-like illness and were euthanized by post challenge day 21. In contrast, all vaccinated dogs remained completely healthy post challenge. Furthermore, vaccinates did not shed CDV at a level detectable by PCR, and passage of nasal and blood samples in susceptible dogs did not result in seroconversion. These results clearly demonstrate that dogs recently vaccinated with one of the most commonly used canine combination vaccines (Duramune® Max5) were protected against multiple virulent isolates of CDV, even when challenged intravenously.

086P
Infectious canine vaccines can cause PCR diagnostic tests to be positive for up to three weeks post vaccination.
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[1]University of Wisconsin-Madison, Madison, WI, USA, [2]Wisconsin Veterinary Diagnostic Laboratory, Madison, WI, USA.

IMMUNOLOGY POSTERS

086P (continued)
PCR is more commonly used today to detect pathogens suspected of causing disease in dogs since it has become commercially available. It is not uncommon for samples for PCR testing (such as nasal swabs, blood, or feces) to be collected after recent vaccination. Most vaccines used in dogs in the US are infectious (attenuated, modified-live) and are given parenterally or locally (intranasal) and are expected to replicate in the immunologically naive animal. The current study was designed to determine if vaccine viruses and/or bacteria are detected by PCR assays, and if so, for how long will the test be positive. We were also interested to see if viruses and bacteria which were not present in the vaccines were detected by PCR in healthy dogs. Results of this study demonstrate that viruses and bacteria in the parenteral and intranasal vaccines can be shed for variable periods of time, up to 3 weeks in blood, nasal secretions and/or fecal samples, thus causing samples to be positive by PCR. These results must be considered when using PCR for the diagnosis of disease in a recently vaccinated animal. Less sensitive testing (ELISA) is recommended for diagnosis of CPV-2 disease in the recently vaccinated dog, as that test has been shown by our laboratory to not pick up low levels of virus as seen after vaccination.

087P
Do current canine combination vaccines provide protection against challenge with multiple isolates of Canine Distemper Virus (CDV)?
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University of Wisconsin-Madison, Madison, WI, USA.

Over the past 5 years it has been suggested that current canine distemper virus (CDV) vaccines do not provide protection from all variants of CDV in the US. This assertion stemmed from observations of CDV outbreaks in a few pet stores and animal shelters only. To date, no studies have been done to demonstrate significant antigenic differences among the genotypic variants of CDV, nor have any challenge studies been conducted. To determine the validity of this claim, we performed a challenge study using 8 dogs which had been vaccinated with modified live CDV (Onderstepoort) or recombinant CDV vaccine 6 to 7 years previously and a group of 3 unvaccinated control dogs, all of which were held in a CDV-free environment. All dogs were challenged intravenously with a CDV cocktail containing 12 different isolates collected from CDV outbreaks around the US, including some from dogs that had been vaccinated but developed CDV disease, and from raccoons that died from CDV. After challenge, dogs were monitored daily for clinical signs, and blood, nasal and rectal swabs were collected to detect challenge virus. Of the dogs which had not been vaccinated, all developed severe distemper-like illness and were euthanized by post challenge day 21. In contrast, all vaccinated dogs remained completely healthy post challenge. These results clearly demonstrate that dogs vaccinated with either of two of the most commonly used canine combination vaccines were protected against multiple virulent isolates of CDV, even when challenged intravenously 6 to 7 years after vaccination.

088P
Animal anthrax spore vaccine with nanogold.
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State Scientific Control Institute of Biotechnology and Strains of Microorganisms, Kyiv, Ukraine.

Purpose: There are many anthrax vaccines licensed in the world. Some of them contains different adsorbent, some not. Mainly all of them are made using nonecapsulated strains. We wished to develop anthrax spore vaccine with nanoparticles. The particles of choice were gold.
Methods: Its biosafety were evaluated by determining their genotoxicity by “alkaline gel electrophoresis of isolated cells”. Also we researched the process of interaction of Bacillus anthracis Sterne 34F2 and nanoparticles of gold with the method of electron microscopy.
Results: After the producing of media with gold nanoparticles the crop of Bacillus anthracis increased in 2-6 logarithms. We developed vaccine with gold nanoparticles which were added to spore suspension of Bacillus anthracis Sterne 34F2. The concentration of gold was 19±2 mkg/ml. As a reference vaccine we used the same vaccine, but without nanoparticles. Vaccine’s potency research showed, that the vaccine with gold particles protected 96,0±0,6 % of animals and the reference vaccine protected 84,0±0,6 %. New product was tested on the laboratory animals and also on cattle, sheep, pigs and horses.
Conclusions: It was totally safe and stimulated animals organism’s to create immunity.

RESPIRATORY DISEASES POSTERS

089P
A survey of porcine reproductive and respiratory syndrome in wild boar populations in Korea.
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No information is currently available on porcine reproductive and respiratory syndrome virus (PRRSV) infection in wild boars (*Sus scrofa*) in Korea. In this study, the status of PRRS in wild boars was investigated. Blood samples were collected from 267 wild boars from 8 provinces in Korea. The samples were tested for antibodies and antigens to PRRSV using a commercial enzyme-linked immunosorbent assay (ELISA) kit and a reverse transcriptase-polymerase chain reaction (RT-PCR) assay to detect PRRSV and to differentiate two genotypes. Four (1.5%) and 8 (3.0%) of the samples tested were positive for PRRS antibodies and antigens, respectively. Of the virus positive samples, 3 and 5 samples were typed as European (EU, type 1) and North American (NA, type 2) viruses, respectively. Two amplicons (one from type 1 and one from type 2) were used to analyze the sequences of open reading frame 7 of PRRSV. The type 1 PRRSV ORF7 nucleotide sequences of wild boar had identities between 96.1 and 98.4% with those of domestic pig-derived PRRS viruses in Korea. The type 2 PRRSV ORF 7 sequences of wild boar had identities of 100% with those of PRRS virus VR2332 strain. The homologies of deduced amino acid sequences of ORF7 between wild boar-type 1 virus and Lelystad virus strain was 92.2%. These results show that PRRSVs are present in wild boars in Korea, and therefore, effective PRRSV surveillance in wild boar population might be useful in disease control.

RESPIRATORY DISEASES POSTERS

090P

Detection of porcine reproductive and respiratory syndrome virus using a Mie scattering immunoagglutination assay in a microfluidic chip.

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A microfluidic immunosensor utilizing Mie scattering immunoaggultination assay was developed for rapid and sensitive detection of porcine reproductive and respiratory syndrome virus (PRRSV) from lung tissue samples of domesticated pigs. Antibodies against PRRSV were conjugated to the surface of highly carboxylated polystyrene microparticles (diameter = 920 nm) and mixed with the diluted PRRSV tissue samples in a Y-shaped microchannel. Antibody-antigen binding induced microparticle immunoagglutination, which was detected by measuring the forward 45° light scattering of 380 nm incident beam using microcallipered, proximity fiber optics. For comparison, multi-well experiments were also performed using the same optical detection setup. The detection limit was determined to be 10-3 TCID50 ml-1 for PRRSV dissolved in PBS, while those of previous RT-PCR studies for PRRSV were 101 TCID50 ml-1 (conventional assays) or <1 TCID50 ml-1 (quantitative real-time assays). Mie scattering simulations were able to predict the shape of the PRRSV standard curve, indicating that any non-linearity of the standard curve can be interpreted purely as an optical phenomenon. Each assay took less than 5 min. A strong correlation could be found between RT-PCR and this method for the lung tissue samples, even though their respective detection mechanisms are fundamentally different (nucleic acids for RTPCR and virus antigens for light scattering immunoagglutination assay). Several different dilution factors were also tested for tissue samples, and 1/10 and 1/100 were found to be usable. If the microfluidic chips are used only once (i.e. without re-using them), both superior sensitivity and satisfactory specificity can be demonstrated. This work demonstrates the potential of the Mie scattering immunoassay on a microfluidic chip towards real-time detection system for viral pathogens in domesticated animals.

091P

Effect of temperature and relative humidity on UV_{254} inactivation of airborne PRRSV.

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Purpose: In the last decade, researchers have verified the occurrence of airborne transmission of PRRSV over significant distances. The use of ultraviolet (UV_{254}) for the inactivation of airborne PRRSV is appealing due to the low cost of implementation relative to filtration, but data on the efficacy of the method is lacking. The objective of this experiment was to evaluate the UV_{254} inactivation of airborne PRRS virus under a range of temperature (T) and relative humidity (RH) conditions.
Methods: Airborne PRRSV was exposed to one of 4 levels of UV_{254} under 9 defined conditions of T (≤15°C, 16°C to 29°C, ≥30°C) and RH (≤24%, 25% to 79%, ≥80%). Samples of air collected after UV_{254} treatment were titrated for infectious PRRSV and the data used to calculate the UV_{254} inactivation constants and UV_{254} half-life (T 1/2) exposure doses for each combination of T and RH. Note: "inactivation constant" is the absolute value of the slope of the line describing the relationship between the survival fraction of the microbial population and UV_{254} exposure dose.
Results: The effects of UV_{254} dose, T, and RH on PRRSV ($TCID_{50}$) recovered in air samples were all statistically significant ($p < 0.001$), as were the interactions between UV_{254} dose*T ($p = 0.0475$), and UV_{254} dose*RH ($p = 0.0204$).
Conclusions: The derivation of inactivation constants and their associated UV_{254} half-life doses for specific conditions of T and RH will make it possible to calculate the UV_{254} dose required to inactivate airborne PRRSV under a range of laboratory and field conditions.

092P

The regulatory role of the zinc finger motif of the PRRSV Nsp1α protein for IFN modulation.

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Non-structural protein (Nsp) 1α of porcine reproductive and respiratory syndrome virus (PRRSV) is an IFN-β antagonist manipulating the RIG-1 singling pathway. Nsp1α inhibits the IκBα phosphorylation and blocks the NF-κB nuclear translocation, resulting in the suppression of IFN-β production during infection. To study the structure function relationship of Nsp1α, eight progressive deletion mutants were made and examined for their ability for IFN induction after poly[I:C] stimulation in a reporter assay. Nsp1α-Δ1-40, Nsp1α-Δ20-60, and Nsp1α-Δ40-80 exhibited high induction of IFN-β activity and showed that the N-terminal one third of Nsp1α contains a crucial element for IFN-β down-regulation. Nsp1α contains two zinc finger motifs, ZF1 and ZF2, to tetrahedrally coordinate zinc ions using C8, C10, C25 and C28 for ZF1 and C70, C76, H146 and M180 for ZF2. To study the importance of zinc finger motifs for IFN-β modulation, eight mutations were individually introduced as below to destroy the motifs: C8S, C10S, C25S, C28S, C70S, C76S, H146Y and M180I. All but C28S suppressed IFN-β induction through both the IRF3 and NF-κB pathways. To avoid a possibility of incomplete destruction of the motifs, double mutations were introduced to each of ZF1 and ZF2. The mutants impairing ZF1 did not suppress the IFN-β production while all mutants disrupting ZF2 retained the IFN-β suppressive activity suggesting that ZF1 is the element important for suppression of IFN-β production. To further confirm the importance of ZF1, triple- and quadruple-mutations were introduced to ZF1 and ZF2 to construct C8S+C70S+C76S, C8S+C70S+H146Y, C8S+C70S+M180I, C8S+C76S+H146Y, C8S+C25S+C70S, C8S+C25S+C76S, C8S+C25S+H146Y, C8S+C25S+M180I, C8S+C25S+C70S+C76S, C8S+C25S+C70S+H146Y, C8S+C25S+C70S+M180I, and C8S+C25S+C76S+H146Y, and their ability for IFN modulation was examined. The mutants with double mutations at ZF1 reversed the IFN-β inhibition, and the results were consistent with the findings from deletions and double mutations studies. Taken together, our data show that ZF1 in the N-terminal region of Nsp1α participates in the IFN regulation during PRRSV infection.

093P

Establishment of a stable cell line expressing non-structural protein 11 of PRRSV.

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Porcine reproductive and respiratory syndrome virus (PRRSV) non-structural protein 11 (Nsp11) has been reported as a type I Interferon antagonist, but how it modulates the interferon production is still unknown. In an attempt to investigate the molecular action of Nsp11, a stable cell line expressing PRRSV Nsp11 was constructed using a retroviral gene transfer system. The Nsp11 coding sequence was cloned from the FL12 North American strain of PRRSV and inserted into the retroviral expressing vector pLNCX2. The plasmid was then co-transfected with pVSV-G into the pantropic packaging cell line GP2-293 to produce a packaged infectious virus containing the Nsp11 gene. MARC-145 cells were infected with the Nsp11-gene containing virus in the presence of Geneticin and cell colonies resistant to the drug were selected and amplified. The Nsp11 gene integration and expression was confirmed by PCR, RT-PCR, and immunoprecipitation. When these cells were examined for IFN induction by poly(I:C) stimulation, the interferon promoter activity was inhibited. Our results indicate that the newly established MARC-145 cells constitutively express the functional Nsp11 protein. This cell line may be a useful tool to study the function of Nsp11.

RESPIRATORY DISEASES POSTERS

094P

Different immunobiological features of two genetically distinct type 2 PRRS Viruses.

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Although it has been generally accepted that porcine reproductive and respiratory syndrome virus (PRRSV) induces weak and delayed protective immunity after infection, it is still unclear that the same immunological concept can be applicable to all PRRS viruses because huge genetic variation exists even in the same genotypes of PRRSV (type 1 or type 2). In the current study, two genetically distinct type 2 PRRS viruses (A and B) which showed approximately 90% nucleotide homology based on ORF5 sequences were characterized based on both *in vivo* and *in vitro* assessments to determine the immunobiological features of the viruses. For *in vitro* assessment, porcine alveolar macrophage (PAM) collected from 3-week old PRRSV-free pigs were infected with each of the two viruses at $1x10^3$ $TCID_{50}$/ml then supernatants and cells were collected separately to determine the relative expression level of IL-1, IL-10, TNF-α, and INF-α/β. In addition, five PRRSV-free pigs were inoculated with either of A or B virus for *in vivo* assessment. At 6 week post inoculation, the pigs were crossly inoculated with each of the viruses to assess the level of cross protection between the viruses. Serum and whole blood samples were collected before virus inoculation and every week thereafter until 8 weeks post inoculation. The serum samples were analyzed for the levels of viremia, nucleocapsid-specific antibody and virus neutralizing antibody. PBMC was also prepared from the whole blood samples and cultured with each virus for 24 hrs to determine the expression level of IL-1, IL-4, IL-10, IL-12, and IFN-γ. B virus induced a significantly higher level of neutralizing antibody and protection parameters as compared to A virus whereas B virus induced a significantly lower level of IL-10 expression than A virus. These results indicate that genetically distinct PRRS viruses could have different immunobiological features and defined immunobiological features could be used to classify various PRRS viruses. The genes responsible for the different immunobiological feature between the two viruses need to be determined in the future.

095P

Development of DNA vaccine against H1N1 subtype swine influenza viruses.

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Purpose: Swine influenza virus (SIV) is an important viral pathogen in pig populations. However, commercial vaccines cannot provide complete protection with induced humoral immunity only and require frequent updates to fight against current isolates. DNA vaccination is an effective means of eliciting both arms of immune system, humoral and cellular immune responses. Therefore, DNA vaccine against SIV was developed in this study.
Methods: In this study, DNA vector pcDNA3.1 was inserted with a chimeric intron downstream of the CMV promoter region followed by a Kozak sequence to enhance the expression of gene inserts. The C-terminal of VP22 gene (VP22c), encoding the tegument protein of bovine herpesvirus-1, was fused separately to the N-terminal of four quadruplicated epitopes, two B-cell epitopes (HA and M2e), and two T-cell epitopes (NP1 and NP2), which were conserved at least among H1 SIV isolates. Linkers -KK- was used to space between each copy of the two B-cell epitopes and -RVKR- was used for the two T-cell epitopes in order to enhance the presentation of epitopes to the immune system.
Results: The expression of epitopes was confirmed in *in vitro* transfection of 293FT cells and higher numbers of epitope-positive cells were achieved from those containing VP22c than those without. After the DNA plasmids were administered to mice intramuscularly in combination or separately, or boosted with recombinant proteins of quadruplicated epitopes fused to VP22c, the vaccine stimulated desired epitope-specific humoral immunity to the two B-cell epitopes and cellular immunity to the epitope NP1.
Conclusions: Our results indicate that the DNA vaccines with quadruplicated epitopes fused to the VP22c may be a potential strategy in developing universal vaccines against SIV.

096P

Porcine respiratory disease complex assessment using a slaughterhouse lung-scoring method in Korea.

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Purpose: The purpose of this study was to assess the lung lesions and to compare gross findings with histopathological findings in the lungs of slaughter pigs
Methods: The 800 lung samples were collected randomly on slaughter pigs during 8 months in Korea.
The lung lesions in each lobe were classified according to the six stages (0%, 1~10%, 11~20%, 21~30%, 31~40%, ≥41%). Severity of gross lesions of the lung was classified with normal, acute, subacute and chronic lesions. For a comparison between gross lesions and histopathology,the 25 lung tissues were collected randomly from each severity on a right cardiac lobe and stained by H&E for BALT hyperplasia histologically. BALT(bronchus associated lymphoid tissue) hyperplasia was graded as follows : (0) absent; (+) mild diffuse infiltration of lymphocytes; (++) moderate increased diffuse infiltration of lymphocytes and/or presence of a few lymphoid nodules; (+++) marked number of lymphoid nodules; or (++++) extensive number of lymphoid nodules.
Results: The results of gross lesion according to stages were : 0; 7.75%, 1~10; 19.25%, 11~20; 29.75%, 21~30; 17.625%, 31~40; 12.125% and >41, 13.5 respectively.According to severity of gross lesions, the lungs were divided into normal (7%, 56/800), acute (38%), subacute (12%) and chronic (43%). BALT hyperplasia associated with severity of macroscopic lung lesions for normal samples (n=25) were subdivided into absent (40.0%) or mild (36.0%), and the acute samples (n=25) were subdivided into absent (20.0%), mild (60.0%, 15/25), moderate (16.0%) or marked (4.0%). The subacute samles (n=25) were subdivided into absent (8.0%), mild (16.0%), moderate (40.0%), marked (28.0%) or extensive (8.0%), and the chronic samples were subdivided into absent (4.0%), mild (12.0%), moderate (40.0%), marked (28.0%) or extensive (16.0%) respectively.
Conclusions: The most prevalent gross lesion according to stage was 11~20%. In severity, the lungs used in this study were a frequent in chronic stage, and it was shown that BALT hyperplasiawas more extensive in chronic lesion. These results indicate that the assessment of lung lesion of PRDC of slaughtered pigs is chronic type.

097P

An investigation of lung lesions and pathogens associated with porcine respiratory disease complex in Korea.

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Purpose: Porcine respiratory disease complex (PRDC) has an important impact on worldwide swine industry. The most common viral agents involved in PRDC include porcine reproductive and respiratory syndrome virus (PRRSV), swine influenza virus (SIV) and porcine circovirus type 2 (PCV2). It is important to remember that the interaction between pathogens can be an important factor in determining the severity of respiratory disease. Those

097P (continued)
pathogens that alter or modulate the respiratory immune system will have a greater overall impact when multiple pathogens are present (1). The purpose of this study was to investigate association with gross lesions and viral agents including PRRSV, SIV and PCV2 in slaughter pig.
Meterials and Methods: A total of 500 lung samples were collected randomly from slaughtered pigs in Korea during August through December of 2010. The gross lesions were classified according to the five stages (0-10, 11-20, 21-30, 31-40, ≥41, unit =%) and 20 samples were selected to detect viral pathogens from each stage. The lung tissues were collected in the part of right cardiac lobe, tissue samples were homogenated and extracted to DNA for PCV2 and to RNA for PRRSV and SIV (QIAamp® DNA Mini Kit and RNeasy® Mini Kit, QIAGEN). PCV2 and SIV were analyzed by real-time PCR, PRRSV was analyzed by nested RT-PCR.
Results: The gross lesions which were classifed according to five stage were shown in Table 2. Relationship with gross lesions and virus detections was shown in Table 3. Forty-one samples were not detected PCV2, PRRSV and SIV-H1N1 by PCR, whereas 11 samples were co-infected.
Conclusion: In this study, PCV2 was the most detected virus, and the detection rate of PCV2 was the highest in the range of 31-40%. PRRSV was the fewest detected virus, and the number of detected viral agent was similar to the result from any ranges of gross lesions. The detection rate of viral agents was the highest in the range of 31-40% of gross lesion. The more severe gross lesions increased, the higher the detection rate showed. These results indicate that the PRDC associated with viral pathogens is widely distributed in Korea.

098P
Serotype and antimicrobial susceptibility of *Actinobacillus pleuropneumoniae* isolated from pigs in Korea.
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Purpose: The aim of this study was to determine the serotypes, antimicrobial susceptibility and the characteristics of the antimicrobial resistance genes of *A. pleuropneumoniae* isolates from diseased pigs in Korea between 2008 and 2010.
Methods: All *A. pleuropneumoniae* isolates used in this study were obtained from lungs of pigs with clinical signs of pleuropneumonia during the period from 2008 to 2010. A total of 32 isolates were cultured on chocolate agar plates and identified *A. pleuropneumoniae* on the basis of biochemical and morphological test. Each isolate was serotyped by means of polymerase chain reaction method. And the susceptibility of the isolates to 13 antibiotics was determined by disc diffusion test. Oxytetracycline and/or florfenicol resistant isolates were examined for the presence of various tet genes and/or floR gene.
Results: In all the 32 isolates examined in this study, serotype 5 (16 isolates: 50%), 1 (7 isolates: 21.9%), 2 (5 isolates: 15.6%) and 12 (1 isolate: 3.1%) were found. Serotype 5 was the predominant isolate and three isolates could not be typed. Of all tested antimicrobial agents, resistance to oxytetracycline was found in 96.9% of *A. pleuropneumoniae* isolates, followed by amikacin (81.3%), neomycin (68.8%), kanamycin (53.1%), penicillin (50.0%), gentamicin (43.8%), florfenicol (25.0%), ampicillin (18.8%), colistin (9.4%), ceftiofur, trimethoprim/sulfamethoxazole (6.3%), and amoxicillin/clavulanic acid (3.1%). Resistance to enrofloxacin was not detected in any isolate of *A. pleuropneumoniae*. The frequency of oxytetracycline resistant isolates was the highest. Among the 31 oxytetracycline-resistant isolates, tetB, tetH and tetO genes were detected in 22(71%), 8(26%) and 1(3%) isolates, respectively. The floR genes were detected in 8 (100%) of the 8 florfenicol-resistant *A. pleuropneumoniae* isolates.
Conclusions: The prevalence of different serovars of *A. pleuropneumoniae* varies among countries. From this survey, *A. pleuropneumoniae* serovar 5 infections are common in pigs. Among the various tet genes, tetB was the most frequently found, similar to Japan and Spain. And the floR gene was confirmed in florfenicol resistant isolates.

099P
Emergence of a new swine H3N2 and pandemic H1N1 2009 influenza A virus reassortant in two Canadian animal populations: mink and swine.
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In December 2010, an outbreak of respiratory symptoms occurred in a hog farm located in the province of Quebec, Canada. At the site, about 10% of the piglets were coughing. However, no increase of the death rate was observed during this swine flu episode. Preliminary PCR results and partial genome sequencing of the HA and M genes suggested that a novel swine H3N2 (swH3N2) and pandemic H1N1 2009 (pH1N1) influenza A virus reassortant (swH3N2/pH1N1) was the cause of this outbreak. Simultaneously, a similar virus was also identified in a Canadian mink farm, based on partial genome sequencing of the HA and M genes. To confirm the existence of the new influenza A virus reassortant, previous and new H3N2 PCR positive cases were first isolated in 10 days old specific pathogen free embryonated chicken eggs. A total of 6 swH3N2/pH1N1 strains were isolated from pig lungs originating from 6 different locations through November 2010 to January 2011. Afterward, all viral genes of the isolated viruses were sequenced. Sequence analyses revealed that the swH3N2/pH1N1 viral genes were related to the North American swH3N2 triple reassortant cluster IV (for HA and NA genes) and to pH1N1 (for M, NP, NS, PB1, PB2 and PA genes). Furthermore, genetic analyses of the HA gene revealed that the swH3N2/pH1N1 strains clustered within 2 subgroups (named subclusters IVa and IVb), suggesting the existence of antigenic variations. To ascertain this hypothesis, an hemagglutination inhibition assay (HI) was conducted with pig sera, using the 6 swH3N2/pH1N1 isolated strains as antigen. The sera were collected from 6 to 8 weeks old pigs originating from the barn where animals have experienced, in December 2010, the first confirmed swH3N2/pH1N1 flu case (related to subcluster IVa). HI results confirmed the existence of significant antigenic variations between strains of subclusters IVa and IVb ($p<0.05$). This study indicates that a novel H3N2 and pH1N1 influenza A virus reassortant has been circulating, since at least November 2010, in the Canadian swine population. It also suggests that this new reassortant has the potential to cross the species barrier since a similar virus has also been identified in mink.

100P
Gene microarray analysis of bovine bronchial epithelial cells exposed to bovine herpesvirus-1 or *Mannheimia haemolytica* in vitro.
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Purpose. Bovine respiratory disease (BRD) often occurs when active respiratory virus infections (BHV-1, etc) impair resistance to *Mannheimia haemolytica* infection in the lower respiratory tract. We used Agilent bovine gene microarray chips containing 44,000 transcripts to elucidate bovine bronchial epithelial cells (BBECs) responses to BHV-1 alone, *M. haemolytica* alone, or both BHV-1 and *M. haemolytica*.
Methods. BBECs were exposed to either BHV-1 (6 hr), *M. haemolytica* (3 hr), or both BHV-1 and *M. haemolytica* in vitro. Total RNA isolated from 3 biological replicates was used for microarray analysis. In addition, conditioned media was collected from BHV-1-infected BBECs to assess how soluble factors from infected BBECs alter bovine neutrophils.
Results. Microarray analysis revealed differential regulation (>2 fold) of 978 transcripts by BHV-1 alone, 2040 transcripts by *M. haemolytica* alone, and 3500 genes by BHV-1 and *M. haemolytica* in combination. BHV-1 induced expression of Ifn-α, Il-1β, Il-1α, Il-8, and Tnfα was confirmed by RT-PCR. By comparison, *M. haemolytica* treatment produced significantly greater inductions (>10 fold) of several genes, including Cxcl2, Ptx3, Il-6, Il-1α, Serpinb2,

100P (continued)
and Il-8, compared to BHV1 alone. Surprisingly, co-exposure to BHV-1 and *M. haemolytica* resulted in a switch from repression to activation for BHV-1 repressed genes such as CXCL10. Functional analysis of the microarray data revealed alterations in genes involved in the biological processes of cell proliferation (Fgf18, Nfkb1a, Fkb1a,), inflammation (Tnfα, Il-8, Tlr2, Ccl55, Il-1α), cell death (Ccl2, Bdnf, Casp3, Casp4,), leukocyte migration (Icam1, Il16), and cell surface markers (Tlr2, CD40). There was significant enrichment of genes associated with leukocyte function, which is consistent with reported changes in bovine neutrophil viability, adhesion and functional activity following incubation with conditioned media from BHV-1-infected BBEC.
Conclusions. We identify differentially regulated genes in BBECs following exposure to BHV-1 and/or *M. haemolytica*. Further analyses will reveal their influence on neutrophil activity and the pathogenesis of BRD.

101P
Variability of *Mannheimia spp*. in ruminant respiratory disease.
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Purpose: Pasteurelleceae are commensals of ruminant upper respiratory tracts but cause severe necrotizing inflammation upon entry into the lungs. Species in this family include a variety of serotypes and biotypes, resulting in little to no cross-protection between types. In the late 1990's, *Pasteurella haemolytica*, the major cause of peracute to acute fibrinosupperative pneumonia, was reclassified; trehalose-negative strains were transferred to the genus *Mannheimia* and divided into five species. *M. haemolytica* is the primary etiologic agent of pneumonic pasteruellosis in livestock species. *M. granulomatis* causes pneumonia, conjunctivitis, and panniculitis, while *M. varigena* causes mastitis, enteritis, and pneumonia in swine and ruminants. *M. ruminalis* and *M. glucosida* are considered non-pathogenic residents of the rumen and upper respiratory tract, respectively. Extensive biochemical characterization were described to speciate these isolates.
Methods: A review of 118 cases submitted to the California Animal Health and Food Safety, Davis, with *Mannheimia spp.*-associated pneumonia was conducted to amass information on phenotypic characterization and association with disease.
Results: *M. haemolytica* was the most commonly detected species from diseased pulmonary tissue (57/118, 67.3%). Nearly 50% (47/118) of isolates could not be identified to the species level using phenotypic characterization. Partial sequencing of the 16S rRNA gene was not useful in identifying these bacteria to species. Both species in this genus that are considered non-pathogenic (*M. ruminalis* and *M. glucosida*) were identified as the sole agents recovered from pneumonic lungs in five cases. In addition, all of these isolates demonstrated beta-hemolysis on blood agar media, a characteristic that has been associated with the presence of the leukotoxin gene, and therefore pathogenic potential, in this genus.
Conclusions: These results indicate that there may be more genetic transfer between these species than previously identified and that further characterization may be necessary to define pathogenic potential of this bacterial agent.

102P
Isolation of *Rothia nasimurium* from the lungs of CFTR-knockout ferrets modeling cystic fibrosis.
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Introduction: Cystic fibrosis (CF) is the most common life-threatening autosomal recessive condition among people of mixed European descent. It is caused by the mutations in an epithelial chloride channel encoded by the *CFTR* gene. CF affects many organs and leads to an average life expectancy of 35 years. The most life-threatening component of the disease is recurrent respiratory bacterial infections. Animal models for CF have been developed to study the disease, including a ferret CFTR-knockout model. *Rothia nasiumurium* is a gram-positive bacterium that has been isolated from the mice, piglets, and the environment. It has not been associated with disease in animals or humans. Purpose: Characterize bacteria found in lungs from CF model ferrets. Methods: Bacterial culture and identification was performed on bronchiolar-lavage (BAL) samples from multiple CFTR-knockout kits (CFTR-/-) as well as wildtype and heterozygous kits (CFTR+/+ or +/-). Initially representative colonies from selective and non-selective media were selected and identified using conventional biochemical identification systems. A subset of these isolates were further characterized by matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF-MS) and 16 S ribosomal RNA (16 S rRNA) sequence analysis. Results: Various organisms were identified from all groups of kits. Organisms resembling coagulase-negative *Staphylococcus* (CNS) were frequently identified from CFTR-knockout kits. Using MALDI-TOF-MS and 16 S rRNA sequencing, *R. nasimurium* has been identified in CFTR-/- kits, but not in control, wildtype and heterozygous kits (CFTR+/+ or +/-). Conclusion: This investigation suggests that *R. nasimurium* may represent an opportunistic pathogen in CF-affected animals. To the author's knowledge, this is also the first report *of R. nasimurium* being isolated from ferrets.

VECTOR-BORNE AND PARASITIC DISEASES POSTERS

103P

Outbreak of bovine epizootic encephalomyelitis by Akabane virus infection in Korea.

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Purpose: The aim of the present study was to determine whether these outbreaks were associated with AKAV infection and to reveal the epidemiological and histopathological characterizations of the disease.
Methods: Histopathological findings of the brain and spinal cords were performed in brain tissues of affected cattle. Brain and/or spinal cords were evaluated with RT-PCR. In addition, Akabane viral antigens were detected by immunohistochemical examination using rabbit antiserum against Akabane virus strain OBE-1.Akabane viruses were isolated using BHK-21 and HmLu cells from brain and spinal cord samples. Antibodies against Akabane virus in a virus neutralization test were detected in sera samples.
Results: Histopathological findings of the brain and spinal cords revealed the presence of nonsuppurative encephalomyelitis in affected cattle. Nonsuppurative encephalomyelitis was observed in 93 of 116 brains and/or spinal cords (80%). Of 116 brain and/or spinal cords, 101 were evaluated with RT-PCR (87%). In addition, Akabane viral antigens were detected by immunohistochemical examination using rabbit antiserum against Akabane virus strain OBE-1. Fifteen Akabane viruses were isolated from brain and spinal cord samples. Antibodies against Akabane virus in a virus neutralization test were detected in 188 of 205 sera samples (91.7%).
Conclusions: A large-scale epidemic of Akabane viral encephalomyelitis in cattle occurred in the southern part of Korea from late summer to late autumn in 2010. It was initially suggested that the syndrome was associated with several environmental factors such as heat stress. However, Nonsuppurative encephalomyelitis was observed in affected cattle by histopathological findings. AKAV as the cause of the syndrome was eventually identified through several diagnostic methods such as IHC and molecular studies, as in that observed in 2000. This is the first report of a large-scale outbreak of bovine epidemic encephalomyelitis by Akabane virus infection in Korea.

104P

Laboratory-based reporting of cattle diseases in the upper northeast part of Thailand in 2009.

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Approximately 20% of domestic ruminants in Thailand are raised in the upper northeast (NE) region. In this area, animal diseases impact to farmers who often are poor and have limited knowledge of animal diseases. The upper NE Veterinary Research and Development Center (VRDC) is a regional laboratory that provides services for all 10 provinces in the region. Specimens come from passive surveillance (sick/dead/decreased production animal) and active surveillance program. These are the most important source of information for early warning, and for the development for more appropriated control and prevention measures. The objective of this study is to estimate the burden of ruminant diseases in the region using passive and active surveillance data collected by VRDC. Ruminant disease database of passive and active surveillance from 10 provinces in NE region were included. Data were analyzed and presented as descriptive study. Submitted specimens were from 9,560 cattle, 789 buffalo, 88 goat, and 21 sheep herds. Three percents of the specimens belonged to passive surveillance program. At herd level, the first three most common diseases from passive surveillance were fascioliasis (4.6%, 95%CI=1.6-12.7), GI nematodes (4.6%, 95% CI=1.6-12.7) and trypanosomiasis (3.1%, 95% CI=1.6-6.0). GI nematodes and fascioliasis accounted for the highest percentage (5.6%, 95%CI=1.9-15.1) in cattle while clostridial infection was found to be the highest percentage (5.8%, 95%CI=2.0-15.6) in buffalo. In active surveillance program, the first 3 highest positive percentage were Caprine Arthritis Encephalitis (33.3%, 95% CI=6.2-79.2), trypanosomiasis (1.3%, 95% CI=0.6-3.0) and fascioliasis (0.4%, 95% CI=0.1-1.6). Our findings demonstrate that common parasitic diseases remain to be a frequent health problem in the ruminants in the NE region of Thailand. It is important to note that Some diseases were less likely to be detected by passive surveillance such as CAE and brucellosis. Motivation to send the specimen and increasing laboratory diagnosis capability can improve coverage of overall surveillance program. Keywords: ruminant, disease, laboratory, Thailand

105P

A universal quantitative PCR assay for tick-borne Anaplasmatacae infections.

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Purpose: As emerging tick-borne diseases in the United States, Anaplasmosis and Ehrlichiosis are important to learn more about because they are etiologic agents of several zoonotic diseases and because of the wide array of domestic species that serve as potential reservoirs. The purpose of these experiments is to further understand these organisms and their transmittance between species by adapting a universal real-time PCR assay for quantification of these infections. Our hypothesis is that the if we quantify the infection levels in the tissues of animals and ticks, then we can discern their susceptibility to the organism between populations and the times the organism is most likely to transfer.
Methods: The first goal is to be able to quantify these infections in samples of blood and tissues using quantitative real time PCR and developing a standard curve. The second goal is to screen with a 16s rDNA-based PCR assay of domestic and wildlife species to learn the prevalence of the disease and also serves to validate the assay. This will be achieved through the use of several experiments such as quantifying infection levels in blood from experimentally infected cattle, experimentally infected tick tissues, and screening for pathogens in two different species of animals. These techniques will be used so that we can assess the amount of infection in the blood and determine whether that trait has significance in mammals and will become a tool to design further experiments.
Results: To date, the quantitative PCR assay has been constructed, plasmid construct completed, and data compiled.
Conclusions: The assay has been proven to be successful in quantifying *Anaplasma marginale* and *Ehrlichia canis* infections, as well as regular 16s-rDNA based detection of the pathogens.

VIRAL PATHOGENESIS POSTERS

106P

Development of highly pathogenic avian influenza and infectious bronchitis viruses plasmid control positive templates for polymerase chain reaction.

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Purpose: Study purpose was to create plasmid based control templates of HPAI and IB viruses for molecular detection and pathogens typing improvement.
Methods: The study was done under using of conventional PCRs with NSC IECVM in house protocols for IB and HPAI virus and TA-cloning. Efficiency of plasmid DNAs was tested by conventional PCR
Results: We prepared specific cDNA matrixes of infectious bronchitis virus and HPAI viruses. These templates were used for amplification of target sequences of IBV (560 bp of S-protein gene) and HPAI viruses (partial fragment of 580 bp H7 and 487 bp H5), responsible both for detection in agarose gel system and sequencing. After amplification both fragments were purified and inserted in pCRBlunt vectors. Canamicin-resistant clones were obtained by LB-medium selection. Number of positive samples of HPAI viruses was 3 and 7, respectively, of H7 and H5, and 4 for IBV. Colonies screening demonstrated 2, 4 and 2 PCR-specific clones of E.coli with avian RNA-contained viruses' genomic insertions. Stability of the clones was demonstrated in serial passages (n = 7).
Clones were used for plasmid DNA-extraction. Stability of DNA was studied in 1-year period.
Double batches were prepared - one for freeze-drying, and one - for cryopreservation (- 20C). PCR-screening demonstrated in dilutions of DNA 1/10 - 1/10000, that reduction of matrix integrity (PCR was negative) in persevered samples (range of detection line was 1/10-1/500(1/1000 IBV)). Dried samples were demonstrated no integrity losses in the period of study.
Vector DNA was used for PCR protocols optimization. They demonstrated unstable products formation (unspecific bands were described for H7 HPAI virus detection PCR and IBV indication protocol). Normal results - monoproduct formation with specific length were obtained in conditions of touch-down PCR techniques.
The new techniques were used for monitoring works and were implemented in viral distribution screening system.
Conclusions: Developed PCR control templates demonstrated efficiency in vitro after testing in conventional PCR

107P

Antigenic properties of multicomponent inactivated vaccines against highly pathogenic avian influenza and Newcastle disease.

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In Ukraine, using epizootic strains of influenza viruses and Newcastle disease, which are isolated in Ukraine, a three inactivated vaccines against avian influenza and Newcastle disease (H5+H7, H5+ND, H5+H7+ND) were developed. There were studied the antigenic properties, the level of antibody immunity and features of its playing in different poultry species (chickens, ducks, geese, turkeys). While vaccination of poultry vaccine H5 + ND level of antibodies to H5 was 9,22-6,38 log2, to ND virus 12,5-6,28 log2. While vaccination of poultry vaccine H5 + H7 levels of antibodies to H5 was 9,0-6,5 log2, to H7 - 9,3-6,1 log2. When immunization H5 + H7 + ND vaccine level of antibodies to H5 was 6,64-5,5 log2, to H7 - 4,08-3,88 log2, ND - 6,21-7,5 log2.

108P

Expression of recombinant glycoprotein D of *Canine herpesvirus* 1 in bacterial cells.

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Purpose: *Canine herpesvirus* (CHV) is a member of *Alphaherpesvirinae* sub-family, with a host range restricted to the *Canidae*. This virus causes acute and usually fatal infection in litters of puppies and may also be involved in infertility, abortion, stillbirth and birth of weak pups. However, in adult dogs the virus causes mild or subclinical infection of the upper respiratory or genital tract and induces latent infection.
CHV is an enveloped double-stranded DNA virus. Glycoprotein molecules that are involved in virus life cycle are incorporated in virus envelope. Among superficial glycoproteins, particularly glycoprotein D is responsible for adsorption and penetration of the virus into the host cells. The goal of this work was to express recombinant glycoprotein D of the virus.
Methods: The gene fragment coding the glycoprotein D of *canine herpesvirus* 1 was PCR amplified and cloned into plasmid vector and expressed in *Escherichia coli* cells. Recombinant protein was then purified by metalochelating affinity chromatography and used as antigen in immunoblot for the detection of CHV-1 specific antibodies.
Results: Antibody testing was performed on the panel of 100 canine sera. Antibodies against CHV-1 infection were detected in 46% of samples.
Conclusions: Our test proved to be a suitable tool for epidemiological and pathogenesis studies in the case of herpesvirus disease in dogs.This work was supported by IGA VFU Brno, 37/2011/FVL

109P

Monitoring of Newcastle disease and the other Paramyxoviruses among wild birds and poultry in Ukraine in 2000-2010.

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Geographical location, climatic conditions in Ukraine provide in its territory a large number of wild birds during migration, wintering, nesting. Ukraine has a developed industrial poultry and a large number of private households; because of it Newcastle disease control is important. In 2000-2010 antibodies to Newcastle disease in serum and egg yolks were found in 48 species of wild birds, also were found antibodies to different Paramyxoviruses subtypes 2-9. Isolated from chickens velogenic viruses ICIP = 1,56-1,61 belonging to 5 genogroups. From wild birds of different ecological systems - lentohenic, belonging to 2 genogroups. From pigeons there were isolated viruses that belong to 4 genogroup. From wild waterfowl it was isolated PMV-4. It was studied the antigenic properties, thermosensitivities, receptor specificity. Some Newcastle disease viruses were tested for production of inactivated vaccines.

ORAL ABSTRACTS

BACTERIAL PATHOGENESIS

001

Detecting differential proteome expression between pathogenic and commensal Staphylococcus aureus strains using SILAC.

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Staphylococcus aureus is a causative agent of bovine mastitis, food-borne diseases and human skin infections. The emergence of antibiotics resistant strains, production of virulence factors and invasiveness makes this pathogen a concern in the food and health industries. The commensal strains of S. aureus exist harmlessly, inhabiting the skin or mucosal membranes of nearly 20-50% of animal and human population. Whereas, the pathogenic strains produce virulence factors which induces a strong immune response resulting in infection. The differential expression of proteins in the commensal and pathogenic strains is proposed here as the basis for the variation in the pathogenicity of the strains. The identification of the differentially expressed protein using Stable isotope labeling by amino acids in cell culture (SILAC) may serve as a potential vaccine target against S. aureus infections. SILAC is a metabolic labeling approach for the identification and quantification of relative protein expression levels between two or more cell states, which in our study are commensal and pathogenic strains. Four commensal isolates obtained from the bovine's hock, teat and nose, along with five pathogenic strains, including ATCC 27217, obtained from the milk of mastitis affected cows were used in the study. SILAC enables the encoding of diverse cellular proteomes of the isolates in minimal medium, i.e. RPMI 1640, containing light (12C) and heavy (13C) isotopes of lysine. The labeled and unlabeled protein fractions, which include cell wall, cytoplasmic, membrane and secreted proteins, are mixed together, trypsin digested and analyzed by mass spectrometry (MS). The protein identification is carried out using the in-house software (MASCOT). The intensity of MS signals corresponds to the relative protein abundance within each sample strain. The identified differentially expressed protein will serve not only as a model for potential vaccine development, but also in determining its role in pathogenesis and immune modulation.

Keywords: Staphylococcus aureus, Stable isotope labeling by amino acids in cell culture

002

Staphylococcus aureus virulence cluster *agr*- regulated hemolysins dictate bovine polymorphonuclear leukocyte inflammatory signaling.

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Purpose: *Staphylococcus aureus* is a major cause of subclinical and chronic bovine mastitis. This versatile pathogen expresses a repertoire of virulence factors that contribute to enhanced pathogen survival through immune evasion. Polymorphonuclear leukocytes (PMN) are the host's primary defense in the mammary gland during infection. However, *S. aureus* virulence factors such as *accessory gene regulator (agr)*-regulated, α-, or δ-hemolysins may impair host inflammatory responses during infection. The objective of this study was to determine the role of *S. aureus* virulence factors in PMN immune responses during infection. Therefore, we hypothesized that *S. aureus* hemolysin expression is responsible for PMN inflammatory signaling. Stress signaling protein phosphorylation leads to host transcription factor activation and cytokine production. Methods: In order to test our hypothesis, bovine PMN were isolated from whole blood and infected with live or irradiated *S. aureus* strains. Strains included *S. aureus* wildtype, *agr*-, α-, δ-, or α/δ-knockout. Lysates were collected for western blot analysis of signaling proteins JNK, ERK, and MAPK p38. Results: Results showed that irradiated *S. aureus* strains did not induce signaling protein phosphorylation, suggesting that signaling induction is a result of live bacteria and not a bacterial membrane induced response. Live *S. aureus* strains induced JNK phosphorylation. However, ERK and MAPK p38 phosphorylation seemed virulence factor dependent. *S. aureus* α-KO did not phosphorylate ERK and *S. aureus agr*- or δ-KO did not phosphorylate MAPK p38 during infection. Conclusions: This data suggests that expressed hemolysins dictate PMN signaling pathway activity and may influence downstream cytokine production, thereby impairing PMN inflammatory responses. Future research will determine cytokine induction during infection to confirm signaling pathway activity.

003

Profile of vasoactive lipid mediators in *S. uberis* mastitis.

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Mastitis remains the most devastating disease to the dairy industry with over 2 billion dollars in annual losses. Prevalence of environmental pathogens, such as *Streptococcus uberis*, has increased in recent years particularly during the periparturient period. *S. uberis* can cause significant tissue damage and long-term production losses, yet the pathogenesis of *S. uberis* remains largely unstudied. Vasoactive lipid mediators, including eicosanoids, are known to play an early role in inflammatory-based diseases by altering vascular permeability and controlling leukocyte infiltration and, dependent upon the eicosanoids produced, promoting enhancement or resolution of the inflammatory response. The objective of this study was to determine the profile of pro-inflammatory molecules, particularly lipid mediators, in *S. uberis*-infected mammary tissue. Three days following *S. uberis* challenge, cows were euthanized and mammary tissue was collected for mRNA and eicosanoid analysis. Increased mRNA expression of cytokines (IL-1β, IL-6, IL-8) in *S. uberis*-infected tissue, when compared to non-infected tissue, was consistent with previous studies. This is the first study to show a significant increase in mRNA expression of vascular adhesion molecules, ICAM1 and VCAM1, suggesting early involvement of the mammary vasculature in the pathogenesis of *S. uberis*. Gene expression of COX2 and 15-LOX1 and production of their downstream eicosanoids, PGE_2/TXB_2 and 15-HETE/13-HODE, respectively, also were significantly increased in the *S. uberis* infected tissue. While changes in COX2 metabolite production have been documented, this is the first study to show involvement of 15-LOX1 in *S. uberis* mastitis. Previous studies have shown the ability of 15-LOX1 metabolites to modulate proinflammatory mediators, including adhesion molecules on bovine endothelial cells. Therefore, subsequent in vitro studies are currently under way to determine to the role of mammary endothelial cells in *S. uberis* pathogenesis. Determining the role of vasoactive lipid mediators in the pathogenesis of *S. uberis* may provide novel therapeutic targets to control the inflammatory response and minimize tissue damage.

004

Metagenomic evaluation of culture-negative clinical mastitis samples.

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Purpose: To better understand the basis for cases of culture-negative mastitis, we performed metagenomic analysis to compare microbial communities in milk taken from culture-negative, clinical mastitis cows to those in milk from non-mastitic quarters from the same animals.
Methods: Total DNA from milk representing pairs of culture-negative clinical and non-clinical samples was isolated from ten animals. Amplicon libraries were prepared using barcoded primers specific to the variable regions 1-2 (V1-2) of the bacterial 16S ribosomal RNA gene and sequenced using a Roche 454 GS FLX instrument with titanium chemistry. Sequence reads were subjected to quality filtering, barcode sorting and 16S rRNA classification.
Results: Data analysis revealed statistically significant differences in composition and abundance of specific bacterial taxa at various phylogenetic levels between the samples. Principle coordinate analysis further indicated differences between clinical and non-clinical non-culturable samples.
Conclusions: Collectively, these results provide evidence that uncultured bacteria are present within raw milk and at significantly different levels in mastitic versus healthy quarters. We propose that these bacteria may contribute to mastitis or may predispose animals to infection by viral or fungal agents.

BACTERIAL PATHOGENESIS

005

Chlamydia pecorum genital infection of dairy cows associates with significantly reduced fertility and decreased levels of circulating luteinizing hormone.

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Chlamydia (*C.*) *pecorum* is routinely detected in cattle but the health impact of these ubiquitous low-level subclinical infections remains poorly characterized. In this study, we analyzed the effect of natural *C. pecorum* on fertility infection of dairy cows. From a 3,700-head dairy farm, 450 randomly selected cows after delivery of the first calf were included in the investigation. From each cow, cervical cytobrush and plasma samples were collected at first breeding. Cows were estrus-synchronized with dual injection of dinoprost tromethamine, and bred by artificial insemination 2 days later. Endpoint for determination of fertility was pregnancy 6 weeks post AI. Cervical samples were obtained immediately after AI by insemination guns modified for cytobrush collection and analyzed by *Chlamydia* 23S rDNA real-time PCR. Luteinizing hormone in plasma was analyzed by radioimmunoassay. Of the 450 cows, 215 (47.8%) were positive in the *Chlamydia* PCR. The detected chlamydial species was in all cases *C. pecorum*. Of the *Chlamydia*-positive cows, 83 (38.6%) became pregnant in the first insemination, and 132 (61.4%) remained open. In contrast, of the 23S *Chlamydia*-negative cows, 112 (47.7%) became pregnant and 123 (52.3%) remained open (p=0.03). Mean plasma luteinizing hormone concentration at AI was significantly lower in *Chlamydia*-positive cows (108 pg/mL) than in *Chlamydia*-negative cows (134 pg/mL; p=0.006). These data confirm the negative influence of asymptomatic *C. pecorum* infection on bovine fertility and a significant effect on production in dairy cows. They furthermore suggest that dysregulation of sexual hormones is at least one of the mechanisms by which chlamydiae influence bovine fertility.

006

Genome scanning for conditionally essential genes in *Salmonella.*

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Purpose: As more whole genome sequences become available, there is an increasing demand for high-throughput methods that link genes to phenotypes, facilitating discovery of new gene functions. In this study, we describe a new version of Tn-seq method involving a modified EZ:Tn5 transposon for genome-wide and quantitative mapping of all insertions in a complex mutant library utilizing massively parallel Illumina sequencing.
Methods: This Tn-seq method was applied to a genome-saturating *S.* Typhimurium mutant library recovered from selection under 3 different *in vitro* growth conditions (diluted LB media, LB media + bile acid, and LB media at 42°C), mimicking some aspects of host stressors.
Results: We identified an overlapping set of 105 protein-coding genes in *S.* Typhimurium that are conditionally essential in one of the above selective conditions. Competition assays using 4 deletion mutants (*pyrD, glnL, recD* and STM14_5307) confirmed the phenotypes predicted by Tn-seq data, validating the utility of this approach in discovering new gene functions.
Conclusions: With continuously increasing sequencing capacity of next generation sequencing (NGS) technologies, this robust Tn-seq method will allow functional scanning of an entire bacterial genome at a high-resolution for a variety of selective conditions of interest. As demonstrated in this study, this Tn-seq method will aid in revealing unexplored genetic determinants and the underlying mechanisms of various biological processes in *Salmonella* and the other approximately 70 bacterial species for which EZ:Tn5 mutagenesis has been established.

007

Exploring APEC virulence, evolution, and host specificity using pathogenomic approaches.

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Colibacillosis, caused by avian pathogenic *Escherichia coli* (APEC), is a costly disease affecting all facets of the poultry industry worldwide. Recent completion of the APEC and chicken genomes has made it possible to use 'systems' and 'genome-wide' approaches to explore the APEC: host interaction as never before. Some of what we thought we knew about APEC pathogenesis has not been verified in these studies, while previously unknown disease mechanisms have been identified. Use of genome-wide analyses of APEC has also demonstrated that carriage of large, transmissible colicin-encoding plasmids typify the APEC pathotype. Such plasmids contain pathogenicity islands and contribute to the pathogenesis of disease in various animal models, including those modeling avian colibacillosis, human urinary tract infections, and human neonatal meningitis. Some of these plasmids may contain resistance islands or co-transfer with multidrug-resistance-encoding plasmids. Thus, use of various antibiotics, disinfectants or heavy metal compounds in poultry production may select for APEC with enhanced capacity to cause disease and resist therapy. Interestingly, evidence exists that these plasmid-containing APEC have emerged only recently. Additionally, comparative analysis of APEC and human extraintestinal pathogenic *E. coli* (ExPEC), such as uropathogenic and neonatal meningitis *E. coli*, has revealed much overlap in their content of virulence traits and abilities to cause disease, stimulating interest in the possibility that APEC is a food-borne source of human ExPEC or a reservoir of plasmid-mediated resistance or virulence genes in human disease.

008

Molecular characterization of Egyptian *Escherichia coli* strains isolated from broiler chickens and their environment.

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Avian pathogenic *Escherichia coli* (APEC), the causative agent of colibacillosis, is responsible for significant economic loss to the poultry industry worldwide, including Egypt. Knowledge of the molecular basis of virulence and antimicrobial resistance of Egyptian APEC strains is scarce. Therefore, this study characterized Egyptian *E. coli* by multiplex PCR to detect eight virulence-associated genes (*cvaC, iroN, ompTp, hlyf, iss, aerJ, ireA* and *papC*), phylogenetic typing, multilocus sequence typing (MLST), serogrouping and antimicrobial susceptibility. Strains were collected from healthy chickens and chickens diagnosed with colibacillosis and their environment from 84 broiler flocks in Egypt. The prevalence of *aerJ* among APEC isolated from heart blood, liver and spleen of diseased broilers was 100%, while the prevalence in isolates recovered from the feces of healthy chickens (AFEC) was 46.44 %. *papC* was detected in 18.18 % of APEC from the liver, while all AFEC were negative. *E. coli* isolated from environmental samples (litter and drinkers) were negative for *cvaC* and *papC*. APEC from heart blood (24/38), liver (30/66) and spleen (16/24), and 14/30 AFEC were assigned to phylogenetic group A, and 15/66 isolates from the liver of diseased birds and 6/30 AFEC isolates were assigned to phylogroup D. Among the 30 APEC strains, isolates were identified as being of the O5, O11, O36, O141, O157 and O158 serogroups, while 13 APEC were nontypeable. AFEC serogroups included O8, O16, O40, O49 and O102 plus two nontypeable strains were found. Screening of *E. coli* strains against 17 antimicrobials using NARMS panels showed that most APEC and AFEC strains were largely resistant to the antimicrobials tested with the exception of amikacin and sulfisoxazole. Multilocus sequence typing of 30 APEC and 20 AFEC identified sequence types 101, 117, 155, 156, 224, 57, 602, 616 and 93 among APEC, and 1011, 162 and 189 among AFEC. In conclusion, there was a high prevalence of virulence genes among APEC and a high prevalence of multidrug resistance in both APEC and AFEC. Egyptian *E. coli* strains show unique sequence types, suggesting the importance of complete characterization of *E. coli* from different parts of the world.

BACTERIAL PATHOGENESIS

009

The siderophore esterases Fes and IroD contribute to virulence of avian extra-intestinal pathogenic *Escherichia coli*.

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Purpose: Extra-intestinal pathogenic *E.coli* is an important pathogen of humans, poultry, and other domestic animals. Siderophores sequester iron and are important for extra-intestinal virulence. In pathogenic *E. coli* the catecholate siderophores enterobactin and salmochelins are degraded by esterases (Fes, IroD, and IroE) to release iron for bioavailability within the bacterial cell. The purpose of this study was to investigate the role of these esterases for virulence of avian pathogenic *E.coli* in the chicken. Growth of strains was also tested in iron poor minimal medium.
Methods: APEC strain X7122 which produces the siderophores enterobactin, salmochelins, and aerobactin was used to generate mutants in the esterase encoding genes fes, iroD, and iroE by using specific allelic exchange of single and multiple esterase encoding genes. These mutants were tested in three-week old chickens via intra air sac inoculation. Bacterial counts were determined in the blood, liver, spleen and lungs 48 hrs post-infection.
Results: Despite the presence of a functional aerobactin system which alone can contribute to APEC virulence, the esterases Fes and IroD were shown to be important for growth in iron poor medium. Loss of catecholate siderophore synthesis in the esterase mutants resulted in a regain in growth. The fes and iroD single mutants were reduced in the blood lungs, liver and spleen. By contrast loss of iroE had no significant effect on bacterial numbers in blood and tissues. Loss of iroD had the most marked attenuation. Cumulative loss of fes, iroD, and iroE did not result in an additive decrease in virulence in the chicken infection model.
Conclusions: Results demonstrate that siderophore esterases IroD and Fes contribute to virulence during systemic infection by APEC strain X7122. As these siderophores systems are highly conserved among APEC and other pathogenic extra-intestinal *E. coli*, esterases may be important targets for inhibiting extra-intestinal virulence in poultry and other animals including humans.

010

Genetic factors affecting the persistence and dissemination of $bla_{\text{CMY-2}}$ positive IncA/C plasmids.

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Purpose: IncA/C plasmids have been isolated from production animals, food products, and clinical isolates. These plasmids carry the $bla_{\text{CMY-2}}$ gene encoding an AmpC-type beta lactamase, and have been observed to persist even in the absence of consistent selection pressure. The cost of plasmid carriage is thought to be related to the extent to which the plasmid can interact with the host genome. Several putative gene products encoded on IncA/C plasmids are predicted to be transcriptional regulators. The aim of this study is to characterize the factors contributing to the fitness cost incurred by a host by IncA/C plasmid carriage.
Methods: Prototype IncA/C plasmid pAR060302 was transferred via conjugation into an *Escherichia coli* host, DH10B. Genes of interest were knocked-out using PCR mediated allelic exchange. Genome and plasmid wide transcription levels of DH10B, DH10B(pAR060302), and DH10B containing deletion mutants of plasmid-encoded transcriptional regulators were measured using deep sequencing technology (RNA-Seq). Fitness cost was assessed by pairwise competition of selected clones and was calculated by the ratio of the number of doublings for the tested clone and the competitor.
Results: Carriage of the plasmid greatly affected host chromosome transcription. Accessory regions of pAR060302 containing the antimicrobial resistance loci were highly transcribed in the absence of antibiotics, including its class I integron and $bla_{\text{CMY-2}}$ loci, whereas much of the core components of the plasmid were not expressed. Deletion of a plasmid encoded H-NS like gene conferred a higher fitness cost to the host than did the wild type plasmid. The transcriptional landscape of the host chromosome was also changed due to the presence of this H-NS like gene.
Conclusions: The relatively unregulated expression of the class I integron and other mobile genetic elements contained on the plasmid likely confer a substantial cost to host. Significant crosstalk occurs between pAR060302 and a host *E. coli* chromosome, and transcriptional regulators carried by pAR060302 appear to orchestrate some of these crosstalk effects.

011

The Cia proteins of Campylobacter jejuni are delivered to the cytosol of host cells via a flagellar-dependent mechanism.

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Purpose: Campylobacter jejuni is a leading cause of bacterial gastroenteritis worldwide. Acute C. jejuni-mediated disease (campylobacteriosis) involves C. jejuni invasion of host epithelial cells that is mediated, in part, by the delivery of the Campylobacter invasion antigens (Cia) to host cells. This study was based on the hypothesis that the Cia proteins are exported via the flagellum of C. jejuni to the cytoplasm of host cells. Methods: To test this hypothesis, we generated a set of mutants with deletions in defined flagellar genes. We then introduced a vector harboring C. jejuni Cia genes fused to the adenylate cyclase domain (ACD) of Bordetella pertussis CyaA into these isolates, and performed assays to detect protein delivery into host cells using an ACD reporter assay. Delivery of the C. jejuni Cia-ACD translational fusion protein to host cells was determined by an increase in cAMP levels, as ACD catalyzes the conversion of ATP to cAMP in the presence of the host protein calmodulin. Results: We found that C. jejuni Cia effectors and flagellar proteins were delivered to the cytosol of host cells, as evidenced by the ACD assay. Secretion and delivery of the Cia proteins required a functional flagellar hook complex (composed of FlgE, FlgK, and FlgL), while the flagellin proteins (FlaA and FlaB) and flagellar cap protein (FliD) were not required. Additional assays revealed that maximal delivery of the Cia proteins was facilitated by the C. jejuni CadF and/or FlpA host cell-fibronectin binding proteins. Treatment of host cells with drugs that prevented C. jejuni uptake into host cells did not affect the delivery of the Cia proteins.
Conclusions: These findings support the hypothesis that the Cia proteins are delivered to host cells following bacterial attachment. To date, this is the only study that demonstrates delivery of C. jejuni effector proteins into the cytosol of host cells.

012

Characterization of the methyl accepting chemotaxis proteins in *Campylobacter jejuni*.

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Purpose: *Campylobacter jejuni* is a major cause of food-borne gastroenteritis in the US and worldwide. *C. jejuni* is a motile bacterium which responds to the extracellular milieu through signal transduction systems consisting of transmembrane methyl accepting chemotactic proteins (MCPs). *In silico* analysis of *C. jejuni* 81-176 genome revealed several putative chemosensory MCPs. MCP proteins have been implicated in proper chemotaxis of bacteria to a specific favorable environment. Further, these proteins also regulate the expression of wide variety of bacterial genes including genes for nutrient uptake, virulence, antibiotic resistance. Therefore, MCPs are considered as an essential prerequisite for bacterial pathogenicity. However, little is known about the mechanisms of *C. jejuni* chemotaxis and the role of individual MCPs in *C. jejuni* pathobiology. The objective of this study was to explore the contribution of methyl accepting chemotaxis proteins to *C. jejuni* pathogenesis/ virulence.
Methods: Deletion mutants of MCPs were generated through homologous recombination and the taxis of these mutants to various chemicals was assessed

012 (continued)
using the modified syringe method. Further these mutants were analyzed for their role in invasion and intracellular survival in INT407 cells.
Results: Several mutant strains were defective in chemotaxis towards chemicals like Fucose, Aspartate and Pyruvate; however 3 strains showed hyperchemotaxis towards chemicals like Formate and L-serine. Furthermore, three mutants were defective in invasion and intracellular survival in INT407 cells.
Conclusion: These results conclude that MCPs play a major role in C. jejuni patho-physiology.

013
In vitro characterization of equine source hyperimmune plasma against *Bacillus anthracis* toxins.
M. Caldwell, K.V. Brock; Auburn University, Auburn, AL, USA.

Abstract Not Available

014
Edwardsiella ictaluri cyclic adenosine 3', 5'-monophosphate receptor protein (crp) mutant vaccine and boost immunization in catfish (Ictalurus punctatus).
J. Santander, R.I. Curtiss; Arizona State University, Tempe, AZ, USA.

Purpose: Edwardsiella ictaluri is an Enterobacteriaceae that causes lethal enteric septicemia in catfish. Being a mucosal facultative intracellular pathogen, this bacterium is a candidate to develop immersion-oral live attenuated vaccines for the catfish aquaculture industry. Boost immunization in the aquaculture industry is not utilized due to the lack of technical approaches and economically accessible vaccines. Deletion of the cyclic 3',5'-adenosine monophosphate (cAMP) receptor protein (crp) gene in several Enterobacteriaceae has been utilized in live attenuated vaccines for mammals and birds. Here we characterize the crp gene, report the effect of a crp deletion in E. ictaluri, and evaluate it as vaccine in fish hosts. Methods and Results: The E. ictaluri crp gene and encoded protein are similar to other Enterobacteriaceae family members, complementing Salmonella enterica Δcrp mutants in a cAMP-dependent fashion. The E. ictaluri Δcrp-10 in frame deletion mutant demonstrated growth defects, loss of maltose utilization, and lack of flagella synthesis. We found that the E. ictaluri Δcrp-10 mutant was attenuated, colonized lymphoid tissues, and conferred immune protection against E. ictaluri infection to zebrafish (Danio rerio) and catfish (Ictalurus punctatus). Evaluation of the IgM titers indicated that bath immunization with the E. ictaluri Δcrp-10 mutant triggered systemic and skin immune responses in catfish. Catfish prime-immersion and oral-boost immunized showed an increase of IgM titers after the oral-boost immunization. Conclusions: We conclude that E. ictaluri Δcrp-10 is an effective immersion-oral live attenuated vaccine that allows the introduction of the oral-boost vaccination in the aquaculture industry.

015
Identification of molecular targets for diagnosis of bovine tuberculosis.
A.L. Lim, J.P. Steibel, S.R. Bolin; Michigan State University, E. Lansing, MI, USA.

Purpose: Previously, we have conducted a microarray-based transcriptional profiling study using antigen stimulated white blood cells (WBC) from cattle that were either infected or not infected with *Mycobacterium bovis*. The purpose was to identify potential molecular markers that could be exploited for diagnosis of bovine tuberculosis (bTB). A total of 281 genes were found that showed altered levels of expression among infected and non-infected cattle (adj $p \leq 0.01$).
Methods: To date, we have validated altered expression for 48 genes, using quantitative real-time PCR (qPCR). For validation, RNA was extracted from antigen stimulated WBC from 3 groups of cattle that either had bTB (n=10) or that did not have bTB (n=26), as determined at postmortem examination or from antemortem test results. Changes in gene expression relative to comparable genes of pooled healthy control animals were computed, and compared among each animal group. Cluster analysis was performed using expression ratios ($-\Delta\Delta Ct$) of differentially expressed genes to cluster bTB infected cattle and non-infected cattle. Linear discriminant analyses were used to select the minimum number of genes required to provide the best separation between groups of infected and non-infected cattle. Predictor model was built using the selected genes and performance of the model was evaluated using 48 cattle of known bTB status.
Results: Twenty four genes had significant differential expression among animal groups, and were used for cluster analysis. Two separate clusters were formed, one cluster contained all but one of the postmortem positive cattle and the other contained all but one of the non-infected cattle. Linear discriminant analyses showed that 7 of those genes were required to provide sufficient statistical power to discriminate cattle with bTB from cattle not infected with bTB. The sensitivity and specificity of the predictor model based on the 7 genes were determined as 95%, and 92.9% respectively.
Conclusions: The gene targets identified in this study are important molecular markers that can be used to develop a rapid and sensitive diagnostic assay for bTB.

016
Genomic signatures of M paratuberculosis from multiple animal species: a better understanding of Johne's disease transmission.
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[1]University of Wisconsin-Madison, Madison, WI, USA, [2]King Abdulaziz City of Science and Technology, Ryiadh, Saudi Arabia, [3]Qassim University, Qassim, Saudi Arabia, [4]King Faisal University, Al-hassa, Saudi Arabia.

Purpose: *Mycobacterium avium* subspecies *paratuberculosis* (*M. ap*) is the causative organism of paratuberculosis or Johne's disease in herbivores and is characterized by chronic granulamatous enteritis with a world-wide prevalence. *M. ap* strain diversity could be responsible for host specificity, distribution and prevalence in different
geographic locations.. Earlier reports described occurrence of Johne's disease in Camels which are important in several regions of the world. However, isolation of a causative agent and identification of disease transmission kinetics remain elusive. Here, we describe our efforts to characterize Johne's disease in camels raised in the Arabian desert, Saudi Arabia and identify the source of infection, especially in a mixed animal breeds farms.
Methods: We have tested >50 clinical samples from infected camels, sheep and goats suffering from classical signs of Johne's disease. The genome of *M. ap* isolates from camel samples were sequenced using Illumina, Next-generation sequencer. Additionally, we used PCR amplifications of IS*900*, *hsp65* and IS1311 targets to genotype infections in
camels, sheep and goats. Finally, whole-genome comparative analysis was used to profile the evolutionary history of *M. ap* in camels.
Results:
We have successfully cultured *M. ap* from several infected tissue samples. PCR amplification of IS*900*, *hsp65* and IS1311amplicons indicated that *M. ap* indeed causes Johne's disease in camel. IS*1311* PCR-REA of camel isolates revealed that isolates belong to Type I, which is more prevalent in sheep.

BACTERIAL PATHOGENESIS

016 (continued)
Genotyping of samples from sheep
and goat further confirmed a sheep source for the camel infection. Further analysis of the camel whole genome identified several regions of insertions/deletions (Indels) as well as thousands of single nucleotide polymorphism.
Conclusion:
Our molecular typing of isolates based on *hsp65*
and IS*1311* sequences revealed that pathogenic Type I [S] strain of *M. ap* is responsible for the Johne's disease in Camels. Despite sheep and goat herds are raised in the same farms with camels, isolates from only sheep were isolated from camels. Our analysis could provide a better control strategy for Johne's disease in camels.

017
Mycobacterium avium subspecies paratuberculosis produces spores.
E. Lamont[1], J.P. Bannantine[2], A. Armien[1], D. Ariyakumar[1], S. Sreevatsan[1];
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Mycobacteria are able to enter into a state of non-replication or dormancy, which may result in their chronic persistence in soil, aquatic environments, and permissive hosts. A clear definition of the mechanism that mycobacteria employ to achieve persistence remains elusive. Purpose: We investigated the potential role of sporulation in one-year old broth cultures of Mycobacterium subsp. paratuberculosis (MAP). Methods/Results: We show that purified MAP spores survive exposure to heat, lysozyme and proteinase K. Heat treated spores are positive for MAP 16SrRNA and IS900. MAP spores display enhanced infectivity as well as maintain acid-fast characteristics upon germination in a well-established bovine macrophage model. Conclusions: This is the first study to demonstrate sporulation in MAP. Data suggest that sporulation may be a viable mechanism by which MAP accomplishes persistence in the host and/or environment. Thus, our current understanding of mycobacterial persistence, pathogenesis, epidemiology and rational drug and vaccine design may need to be re-evaluated.

018
Molecular characterization of the Mycobacteria antigen 85 complex and Fibronectin interaction.
C.-J. Kuo, **Y.-F. Chang**; Cornell. University, Ithaca, NY, USA.

Antigen 85 complex (Ag85), consisting of members 85A, 85B and 85C, is a predominantly secreted protein of mycobacteria and possesses the ability to specifically interact with fibronectin (Fn). Since fibronectin-binding proteins are important virulence factors of Mycobacterium spp., Ag85 may contribute to the adherence, invasion, and dissemination of organisms in host tissue. In this study, we reported the Fn-binding affinity of Ag85 of Mycobacteria avium subsp. paratuberculosis (MAP) (KD was determined from 40-76nM) and mapped the Ag85-binding motifs of Fn. Fn14, a type III module located at heparin-binding domain II (Hep-2) of Fn, was discovered to interact with Ag85 of MAP. Peptide inhibition assay subsequently demonstrated that peptide 14-26 (14TPNSLLVSWQPPR26, termed P14-26) interfered with Ag85 bound to Fn (73.3% reduced). In addition, single-residue substitution of P14-26 revealed the key residues (Leu21, Val22, Ser23, Pro24, Pro25, and Arg26) involved in Ag85-Fn binding, which might contribute to the hydrophobic and ionic interactions. Moreover, the binding of Ag85 on Fn siRNA-transfected Caco2 cells could be dramatically reduced (44.6%), implying the significance of Ag85-Fn interaction between mycobacteria and host cells. Our results indicated that Ag85 bound to Fn at a novel motif and plays a critical role in mycobacteria adherence on host cells to initiate infection, which might play an important colonization factor contributing to mycobacterial virulence.

019
The structure of a calcium-binding leptospiral immunoglobulin-like protein (LigB) domain of Leptospira interrogans.
C.P. Ptak[1], C.-L. Hsieh[1], Y.-P. Lin[1], A.S. Maltsev[2], R. Raman[3], Y. Sharma[3], **Y.-F. Chang**[1];
[1]Cornell. University, Ithaca, NY, USA, [2]National Institutes of Health, Bethesda,, Bethesda, MD, USA, [3]Center for Cellular and Molecular Biology, Hyderabad, India.

A number of surface proteins specific to pathogenic strains of Leptospira have been identified. The Lig protein family has shown promise in typing leptospiral isolates for pathogenesis. We use NMR spectroscopy to solve the solution structure of the twelfth Ig repeat domain from LigB (LigB12). LigB12 has a similar greek key fold to the Ig domains from E. coli intimin and Yersinia's invasion. Identified using both ITC and NMR spectroscopy a calcium binding site stabilizes the fold. A tryptophan is located within hydrophobic core of the fold revealing a potential mechanism for its shifted fluorescence. For the LigB Ig domains, a conserved patch faces the next sequential domain while in LigB12, this region faces the non-repeat region and accordingly diverges from the other 11 repeats. The location of a possible domain-domain interaction surface can be inferred. The LigB12 structure is the first Ig domain structure from Leptospira spp. and should prove useful in understanding host interactions with the Lig protein family.

020
Evaluation of oral fluid samples as a diagnostic sample for detection of *Erysipelothrix rhusiopathiae* in pigs.
L.G.G. Lirola, Jr., P.G. Halbur, T. Opriessnig; ISU, Ames, IA, USA.

Purpose: Swine erysipelas (SE) caused by *Erysipelothrix rhusiopathiae* continues to be an important disease of pigs. The lack of ante mortem tools to detect the circulation of *E. rhusiopathiae* in pig populations makes controlling SE a challenge. *E. rhusiopathiae* is typically diagnosed postmortem and the current gold standard is isolation of the bacteria from tissues of dead pigs. If a change in circulation of *E. rhusiopathiae* could be detected, intervention strategies could be initiated to minimize losses. Oral fluids are being increasingly used for characterization of pig viral pathogens in the population. Methods: To determine if oral fluids are an appropriate sample for *Erysipelothrix rhusiopathiae* detection, 28 pigs were randomly divided into 7 groups with 4 pigs in each group. Twenty of the 28 pigs were inoculated intramuscularly with *Erysipelothrix rhusiopathiae* strain EI-6P, a known pathogenic serotype 1a isolate. After inoculation, the pigs were monitored every 3 hrs for development of fevers and other clinical signs and oral fluids were collected every day from each group.
Results: The majority of the infected pigs developed clinical signs consistent with SE (fever, lethargy, diamond-shaped raised skin lesions) and were treated with ceftiofur intramuscular. *Erysipelothrix rhusiopathiae* was isolated from oral fluids obtained from 1/6 pens containing infected pigs at 24 and 48 hrs after inoculation. In addition, *Erysipelothrix rhusiopathiae* DNA was detected by real-time PCR in 5/6, 3/6, 4/6 and 0/6 of the oral fluid samples from infected pigs at 1, 2, 3 and 7 days post infection.
Conclusions: The results indicate the oral fluids are a good sample for rapid detection of *Erysipelothrix rhusiopathiae* in pigs.

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021

Seroprevalence of brucellosis in sheep in the selected areas of Mymensingh and Netrakona districts of Bangladesh.

M.S. Rahman, M. Ahsan, M. Rahman, N. Jahan; Bangladesh Agricultural University, Mymensingh, Bangladesh.

Brucellosis is an economically important disease prevalent in Bangladesh. Bangladesh has been reported an endemic area for brucellosis. The occurrence of brucellosis in people and the distribution of the prevalence among the livestock to be spatially related. A survey of brucellosis was carried out in sheep of Netrakona and Mymensingh districts of Bangladesh. A total number 102 blood samples were collected from sheep in Mymensingh and Netrakona District of Bangladesh. Information of sheep's age, sex, location, pregnancy status, abortion, reproductive disorder were collected and tested for *Brucella* specific antibody response by RBT and iELISA. The overall serological prevalence of brucellosis was recorded as 5.88% in sheep. The prevalence of brucellosis in female sheep was higher (7.79%) than in male (0.00%). The prevalence of brucellosis in sheep with abortion was 20.00% than sheep with no abortion (7.54%). The higher rate of *Brucella melitensis* antibody was recorded in sheep above 2 years of age (25.00%).The study revealed that the risk of being infected by brucellosis of sheep in Netrakona is 1.54 times higher than that of a sheep in Mymensingh. The result also suggests that female sheep has an increased chance of brucellosis than a male. The observed relationships between *Brucella melitensis* status, sex and age are consistent with what is generally known about the biology of the infection. Higher prevalence also found in animal with a previous abortion record than non aborted animal. Finally, the results of the study provide a baseline data for further study of *Brucella melitensis* infections in the area and a starting point for control measures of brucellosis. Further studies for isolation, identification and characterization of *Brucella* organism are recommended.

022

Identification of antigens for more reliable *Streptococcus equi* specific ELISA assay.

S. Artiushin, S. Velineni, J.F. Timoney; University of Kentucky, Lexington, KY, USA.

Purpose: ELISA based on the full length M-like protein (SeM) of *Streptococcus equi* (Se) is widely used for confirmation of diagnosis and retrospective identification of asymptomatic infections. It is also helpful in diagnosis of Se associated purpura haemorrhagica and bastard strangles. Interpretation of results is sometimes complicated by the presence of antibody to SzM, the homologue of SeM in *S. zooepidemicus* (Sz).
Our hypothesis is that substitution of full length SeM by oligopeptides spanning the N-terminus of SeM or other proteins of *S. equi* will improve specificity of the ELISA.
Methods: Regions of SeM, Se18.9, IdE2, P3, P6 and JP2 proteins specific to Se were selected following comparative genomic analysis of isolates of Se and Sz. These sequences were cloned into pET15b or pET31b vectors. Expressed oligopeptides SeM-N1, SeM-N2, Se18.9-N, IdE2-N, P3-N, P6-N, JP2-N and full length SeM were purified by metal affinity chromatography and compared in ELISA for reactivity with sera from horses exposed to Se or Sz infections.
Results: All oligopeptides except of IdE2-N and P6-N showed strong reactivities with sera from horses recovered from strangles. SeM, Se18.9-N and P3-N were also strongly reactive with 6, 5 and 1of 10 sera from horses with Sz infection suggesting production of crossreactive antigens by corresponding Sz strains. Sz specific sera were unreactive or only slightly reactive with SeM-N1, SeM-N2 and JP2-N while these peptides showed strong reactions with Se specific sera that were comparable to those for SeM.
Conclusions: We conclude that N-terminal peptides of SeM or N-terminal part of JP2 substantially increase the specificity of ELISA for Se antibody, a tool useful in diagnosis of exposure of horses to the pathogen and of metastatic abscessation and purpura hemorrhagica.

023

The role of Th17 cytokines, IL-17A and IL-22, during Listeria monocytogenes infection of the pregnant mouse.

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Purpose: Pregnant women are 20 times more likely than the average adult to acquire invasive listeriosis and represent 16% of reported *Listeria* cases in the USA. The developing fetus is a common target for *L. monocytogenes* infection during pregnancy, frequently leading to abortion, premature birth, and neonatal sepsis. The pathophysiology behind the immune failure in pregnant women infected with *L. monocytogenes* is poorly understood. The increased susceptibility of pregnant women is hypothesized to be due to altered T cell immunity. The roles of T helper cells have long been suspected and Th17 cells have arisen as a potential source of pro-inflammatory mediators that are deleterious to fetal health.
Methods: Pregnant mice at 10-14 days of gestation were infected intragastrically with a serotype 4b strain of *L. monocytogenes* isolated from a foodborne listeriosis outbreak that resulted in abortion and fetal death. Maternal and fetal tissues were harvested for qPCR analysis of cytokine production associated with multiple T cell subsets. We then tested the importance of the major Th17 effector cytokines, IL-17 and IL-22 using IL-17A and IL-22 knockout mice.
Results: Intragastric infection of mice with *L. monocytogenes* resulted in production of IL-17 and IL-22 mRNA in maternal and fetal tissues. However, no significant differences were found in microbial load or tissue inflammation in maternal and fetal tissues of IL-17A or IL-22 knockout mice when compared to C57BL/6 wild type mice.
Conclusions: These data suggestion that TH17 cell effector cytokines are present and could play a role in inflammation at the maternal-fetal interface in the decidual placenta. However, they are part of a redundant inflammatory process and fetal survival during murine listeriosis is not dependent on IL-17A or IL-22.

024

Diversity among clostridial isolates from cellulitis cases in turkeys.

A.J. Thachil, K.V. Nagaraja; Department of Veterinary and Biomedical Sciences, University of Minnesota, Saint Paul, MN, USA.

Clostridium perfringens and Clostridium septicum are recognized as the causative agents of cellulitis in turkeys. Our earlier studies had reproduced cellulitis with either *C. perfringens* or *C. septicum* experimentally in turkeys. The objective of this study was to characterize Clostridial isolates from cellulitis cases of turkeys and also to identify their toxin profiles. In brief, *C. perfringens* and *C. septicum* isolates from cases of cellulitis in turkeys were examined in this study. The isolates were grown in suitable media and DNA was extracted before conducting multi locus sequence typing (MLST). The culture supernatants from these isolates were subjected to two-dimensional gel electrophoresis to separate out the toxins. Suspected proteins were identified by MALDI-TOF mass spectrometry. Our results suggested high diversity among *C. perfringens* from cellulitis cases in turkeys when compared with *C. septicum* isolates. The findings will enable us in selecting vaccine candidates for the development of an effective vaccine against cellulitis in turkeys.

BIOSAFETY AND BIOSECURITY

025

Biosecurity assessment as a tool towards risk-based surveillance on swine farms in southern Ontario.

K. Bottoms, Z. Poljak, C. Dewey, R. Friendship; University of Guelph, Guelph, ON, Canada.

Purpose: The primary objective of this project is to develop a map of southern Ontario that can be used as a tool in the risk-based surveillance of contagious swine diseases. For this purpose, we are interested in external biosecurity protocols. Using cluster analysis, we can determine how many biosecurity groups are present, and which factors are most important in defining these groups. The risk map will incorporate spatial information about the distribution of these biosecurity groups with the density of swine herds. Additionally, multinomial logistic regression will allow us to determine which demographic variables are significant predictors of biosecurity group membership.
Methods: Variables pertaining to external biosecurity practices were selected for cluster analysis, allowing us to determine the best number of groups to describe biosecurity on our sample of farms. Geographic information about farms in each of the identified groups was combined with the density of swine herds in the study region to develop a weighted map that demonstrates areas of risk for the incursion of contagious swine diseases.
Results: The most important factors in distinguishing the identified biosecurity groups were variables pertaining to trucking practices, sanitation procedures, and source of replacement animals. Multinomial regression analysis identified significant predictors of biosecurity group membership. These include herd size, herd type, production type and number of swine herds within a three mile radius of the site.
Conclusions: Geographic areas identified as 'high risk' have high densities of swine and/or a low probability of having herds that are classified as having good biosecurity practices. Surveillance of emerging contagious swine diseases would best be focused in these areas. Alternatively, areas identified as 'low risk' have low densities of swine and/or a high probability of having herds that are classified as having good biosecurity practices. This information, along with the knowledge of significant predictors of biosecurity group membership, can be utilized by the swine industry to maximize the benefits of surveillance projects involving contagious swine diseases.

026

Evaluation study of interventions for reducing the risk of PRRSV introduction into filtered farms via retrograde air movement (Back-drafting) through idle fans.

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Purpose: The objectives of this study were to demonstrate that the entry of PRRSV-contaminated aerosols into a filtered facility via retrograde air through unfiltered points (i.e. idle fans) is a true risk; to titrate the minimum air velocity necessary to introduce PRRSV-contaminated aerosols via retrograde air; and to validate commercially available interventions that have been designed to prevent this risk.
Methods: The study, conducted at the UMN SDEC production regional model site, used an empty facility under negative pressure ventilation. In order to create retrograde air movement through an idle fan, one of the 2 fans was intentionally stopped while the other continued to operate. The retrograde air moved through a common plastic shutter intervention at several fan stages. Each stage was challenged with 10 replicates of different PRRSV concentrations (1 through 7 logs each) which were generated using a cold-fog mister located on the exterior of the facility. A cyclonic collector was used to collect a sample from each replicate. To titrate the air velocity needed to transfer PRRSV, an anemometer was utilized. The measurements of retrograde air velocities and static pressures were collected for each fan stage.
Interventions evaluated included the standard plastic shutter, plastic shutter plus canvas cover, nylon air-chute, aluminum shutter plus air-chute and, double shutter system (aluminum and plastic shutters). All 5 interventions were challenged as described in order to determine whether aerosolized PRRSV could penetrate the different treatments.
Results: The results of this study suggest that a real risk of PRRSV entry may exist when there is a minimum retrograde air speed of 0.76 m/s. As well, this study suggests that the plastic shutter and canvas cover do not offer complete protection against retrograde air movement and the risk of aerosolized PRRSV entry.
Conclusion: Results from this study indicate that unfiltered retrograde air movement is a risk for PRRSV introduction in filtered farms and that it requires a minimum velocity of air flow. Finally, this study confirms that not all interventions designed to reduce this risk are effective.

027

Isolation of Salmonella organisms from the environment in a large animal hospital using electrostatic (Swiffer) and sterile sponge collection devices.

A. Ruple, B. Burgess, P.S. Morley; Colorado State University, Fort Collins, CO, USA.

Purpose: The objective of this study was to compare methods of detecting environmental contamination with Salmonella organisms in a large animal veterinary teaching hospital.
Methods: Environmental samples were collected using both electrostatic and sponge collection devices from stalls used to house either horses or cows that were confirmed to be shedding Salmonella organisms by fecal culture. Samples were obtained from stall sites after cleaning and disinfection had been completed. Areas within the stall that were sampled included floors, walls, and feed and water containers, with a similar amount of surface area covered with both sampling devices. Multiple enrichment protocols were used for bacteriologic culture of samples obtained using each of the sampling devices.
Results: A total of 100 paired environmental samples were collected and bacteriologic culture results were concordant in 88% of the samples. Of the paired samples with discordant bacteriologic culture results, Salmonella organisms were detected in the sample collected with the electrostatic collection device in 11 out of 12 (91.7%) of the samples.
Conclusions: These results suggest that the sampling and culture method used with the electrostatic collection device is more sensitive than is the sampling and culture method used with the sterile sponge collection device.

028

A novel animal product-free avian cell line that supports high titer influenza virus replication.

M. Carvajal-Yepes, K.A. Smith, T. Almy, C.J. Colvin, P.M. Coussens; Michigan State University, East Lansing, MI, USA.

The current most common method of growing influenza virus vaccines is through use of "clean" embryonated chicken eggs. This manufacturing system selects for receptor-binding variants with reduced antigenicity and requires significant downstream processing for purification. Several manufacturers are moving toward a cell culture-based vaccine production system, which reduces the possibility for contamination and promises to be more reliable, flexible and expandable than egg-based methods. This process and regulatory approvals have been slow due to concerns that most continuous cell lines are tumorigenic and require addition of tosyl phenylalanyl chloromethyl ketone (TCPK)-trypsin, to release sufficient virus for vaccine production. Based on numerous derivations of an existing cell line, CHCC-OU2, we have developed a novel immortalized non-tumorigenic chick embryo cell line, termed PBS-12SF, which is capable of growing unmodified recent isolates of human and avian influenza A and B viruses to extremely high titers (>107 PFU/ml) without the need for exogenous proteases or serum. PBS-12SF cells express receptors for both human and avian influenza. Typically, FDA requires that vaccine viruses remain genetically stable during scale-up for vaccine production. Using PCR and multiple sequence alignments of the HA and N genes, we

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028 (continued)
are examining stability of two recent influenza virus vaccine strains serially passed in PBS-12SF cells. Cellular defenses against viral infections are typified by production of Interferon alpha and beta (IFNα/β). IFNα/β bind to IFN alpha-beta receptor (IFNAR) on the cell surface and induce an antiviral state, limiting viral replication. Despite high titers already observed in PBS-12SF cells, there is a robust interferon (IFN) response to influenza virus infection in these cells, particularly IFNβ. We hypothesize that influenza, and other viruses shown to replicate on PBS-12SF cells, will achieve even higher titers if the IFN α/β response is neutralized. Thus we are developing a stable PBS-12SF cell line in which we have knocked down the interferon pathway, by using short hairpin RNAs (shRNAs) specific for the interferon alpha/beta receptor (IFNAR).

029
Development of multiplex-PCR and electronic microarrays for detection and typing of avian and bovine high consequence viruses.
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Accidental or intentional introduction of exotic high consequence (HC) pathogens of livestock and poultry can have catastrophic consequences on agriculture industry. Early detection of HC pathogens is the key to prevent and/or control such an event. Electronic microarray technology utilizes electric field control to facilitate analytes concentration, hybridization and stringency providing unique features of speed, accuracy and multiplexing capability for genomic analysis. Combining multiplex-PCR with electronic microarray technology, we have developed independent assays to detect and type bovine and avian HC viruses. Using the NC400 amplicon-to-answer electronic microarray instrument, we have demonstrated that the bovine HC assays simultaneously detect and differentiate 8 viruses which include 4 bovine HC viruses and their differentials and distinguish all 7 foot and mouth disease virus (FMDV) serotypes. The avian HC assays simultaneously detect and differentiate avian influenza virus (AIV) and Newcastle disease virus (NDV), pathotype NDV and distinguish all 16 hemagglutinin (HA) subtypes and all 9 neuraminidase (NA) subtypes of AIV.
The bovine assays accurately detected and typed 23 virus strains representing all seven FMDV serotypes and detected and differentiated 37 strains of bovine HC viruses. Viruses from all seven FMDV serotypes were detected as early as 2 days post infection in both oral and nasal swab materials from experimentally infected cattle. The avian assays accurately detected and typed 41 AIV strains representing all HA and NA subtypes, and 22 NDV strains representing both high and low pathotypes.
The assays developed for NC400 are being adapted to a new portable, fully-automated, sample-to-answer instrument that can be operated on the farm site. The sample-to-answer instrument, unlike the NC400 instrument, uses inexpensive carbon micro-electrode arrays. To date, we have successfully identified and subtyped 10 bovine and avian viruses using the new sample-to-answer instrument which minimizes the labor and the skill level required by the end-user and allows rapid multiplex on-farm testing.

030
Working with Biolevel 3 Agents that interface across human, livestock, and wildlife boundaries.
S. Olsen; Infectious Bacterial Diseases of Livestock Research Unit, National Animal Disease Center, Ames, IA, USA.

Biocontainment and regulatory requirements for work with pathogenic viruses, fungi, and bacteria in the U.S. continue to increase. Brucellosis and tuberculosis are examples of zoonotic pathogens of economic importance that are classified as biocontainment level 3 agents (BL3) and endemic in domestic livestock and wildlife hosts in the U.S. Billions of dollars have been invested in regulatory programs by federal, state, and producers to achieve the current status of disease control for these zoonoses. In this presentation, experiences and approaches for conducting research studies with zoonotic pathogens in natural hosts (domestic livestock and wildlife) under BL3 conditions will be discussed. Although there are safety risks to be managed, species differences in vaccine responses and disease pathogenesis dictate that research be conducted in the relevant host species. Species-specific characteristics were considered in developing work practices for wildlife and domestic livestock and in design of containment facilities. Our experience indicates that handling and containment risks can be managed and the research studies safely conducted. These types of studies are vital for regulatory personnel in their efforts to design strategies to control or eradicate zoonoses in natural hosts, protect public health, and protect the economic investment made in national control or eradication programs.

COMPANION ANIMAL EPIDEMIOLOGY

031
Prevalence and risk factors for shedding of *Campylobacter* spp. in dogs that frequent dog parks in southern Ontario, Canada.
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The prevalence of, and risk factors for, shedding of *Campylobater* spp. in feces of dogs that visit dog parks in southern Ontario were examined. From May to August of 2009, dog fecal samples were collected at ten dog parks and owners were asked to complete a survey relating to pet management factors, such as diet, exposure to other pets and livestock, treatment with antibiotics, and human illness in the home. A total of 251 fecal samples were collected and 189 surveys completed. *Campylobacter* spp. were found in 108 (43%) fecal samples. Of the *Campylobacter* spp. detected, 86.1% were *C. upsaliensis* while 13.8% were *C. jejuni*. Multivariable logistic regression models were created to identify risk factors for a dog testing positive for any *Campylobacter* spp. and *C. upsaliensis*. Factors significantly associated with shedding of *Campylobacter* spp. included access to surface water for swimming or drinking, such as lakes and ditches. Sparing factors included increasing age of the dog, feeding of a commercial dry diet, and exposure to compost. These factors were also statistically significant when examining *C. upsaliensis* specifically, except exposure to compost which was not statistically significant. Age as a sparing factor was expected as young animals have been reported to shed *Campylobacter* spp. more frequently than older animals. The feeding of a commercial dry diet as a sparing factor may result from those dogs being less likely to consume raw or cooked homemade diets, which have been reported as risk factors in previous studies. Access to water as a risk factor may be due to fecal contamination of the water by other animals including dogs. Exposure to compost as a sparing factor in the *Campylobater* spp. model may be due to correlations with an unmeasured management factor, but this effect should be examined in further studies.

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Comparison of virus isolation, one-step real-time reverse transcriptase-PCR assay, and two rapid influenza diagnostic tests for detecting canine influenza virus (H3N8) shedding in dogs.

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Purpose: The widespread circulation of canine influenza virus (CIV) in the U.S. dog population has established influenza as a threat to canine health. As rapid and accurate detection of infection is a critical factor in the diagnosis and control of CIV, a one-step real-time reverse transcription (rRT)-PCR assay, based on the influenza virus matrix (M) gene, was recently developed by our laboratory for quantification of influenza virus from swab samples. The objective of this study was to compare traditional virus isolation (VI), the rRT-PCR assay, and two rapid influenza detection tests (RIDT) in detecting CIV.
Methods: Nasal and pharyngeal swabs were collected from 122 shelter and household dogs seen at Colorado State University Veterinary Teaching Hospital (CSU VTH) for canine infectious respiratory disease between April 2006 and March 2007 and from 1372 dogs housed in six U.S. shelters from December 2009 to November 2010. For the CSU VTH samples, a stochastic latent class analysis modeling approach was used to estimate sensitivities and specificities of VI, the rRT-PCR, and the Directigen Flu A+B® (DFA) RIDT in detecting CIV shedding in dogs. To determine significance between cycle threshold values among samples and between shelter and household dog populations, two-tailed Student-t tests and one-way ANOVA were calculated. Additionally, from the shelter dog samples, sensitivity, specificity, positive predictive value, and negative predictive value were determined using traditional calculations from a 2x2 contingency table for the Flu Detect® (FD) RIDT.
Results: The median sensitivities of VI, DFA, and the rRT-PCR were 47-80%, 53-70%, and 94-96%, respectively. Median specificities ranged from 95-98% for VI, 91-96% for DFA, and 74-87% for rRT-PCR. There was no statistical difference for mean cycle threshold values between nasal and pharyngeal swabs or between shelter and household dogs. Furthermore, the FD test performed poorly for detecting CIV compared to rRT-PCR in shelter dogs.
Conclusions: In conclusion, rRT-PCR has the highest sensitivity among the testing methods we evaluated for detecting CIV and is an appropriate test for rapidly diagnosing CIV shedding in dogs.

033

Comparison of the geographical distribution of Feline Leukemia virus (FeLV) and Feline Immunodeficiency virus (FIV) infections in the United States (2000-2011). **B. Chhetri**, O. Berke, D.L. Pearl, D. Bienzle; University of Guelph, Guelph, ON, Canada.

Purpose:It has been suggested that FIV and FeLV incidence rates have different geographic distributions in North America even though both infections share similar known risk factors and control measures. This exploratory analysis investigated the geographical distribution and clustering of both viral infections with respect to each other in the 48 contiguous U.S. states. If underlying known or unknown risk factors for respective infections vary in space, then we would expect to see regions with excesses of one infection over the other.
Methods: Counts of FIV (n=17,108) and FeLV (n=30,017) positive serological tests (FIV antibody and FeLV ELISA) were obtained for each U.S. state from the IDEXX Laboratories' website. The ratio of FIV to FeLV infection was estimated for each state and a choropleth disease map was used to visualize the spatial pattern. The statistical significance of spatial clustering of each infection relative to the other was estimated using the spatial scan statistic with a Bernoulli model.
Results:In this study, FIV and FeLV had distinct spatial distribution patterns. The choropleth disease map revealed higher numbers of reported FIV infections in the Southern and Eastern U.S. compared to FeLV. In contrast, reported FeLV infections were observed to be higher in the Western and North-Central U.S. compared to FIV. Statistically significant spatial clusters located in the Western US for FeLV and the Southern and Eastern US for FIV confirmed these results.
Conclusions: The observed variability in geographical distribution of these retroviruses relative to each other may be related to the differential distribution of additional risk factors including those related to different strains of these viruses, the prevalence of vaccine use for FeLV, cat demographics (e.g., outdoor access and population density) and other contextual risk factors that may vary in space and affect pet health management (e.g., human demography and socio-economic factors).

035

Prevalence of and exposure factors for H3N8 canine influenza virus seropositivity in US dogs with influenza-like illness.

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Purpose: Knowledge of H3N8 canine influenza virus (CIV) seroprevalence and factors associated with seropositivity in US dogs is limited. Reported seroprevalence estimates of H3N8 CIV in dogs with or without influenza-like illness (ILI) have ranged from 0.5 to 42%. Attending canine-day-care, number of days in a shelter, and multi-dog households have been associated with seropositivity. However, reported studies were limited to one geographic location and/or used small sample sizes. The objectives of this study were to estimate the seroprevalence of H3N8 CIV in a sample of US dogs with ILI, and to identify exposure factors associated with seropositivity.
Methods: This cross-sectional study utilized sera from 1,268 dogs with ILI in 42 states. Canine sera collected during 2005-2009 were tested for CIV H3 antibodies using the hemagglutination inhibition assay. The frequency of intrinsic factors (age, breed, sex), exposure settings (shelter facilities, boarding kennels, other settings), and geographic region (Southwest, West, Midwest, Southeast, Northeast) was compared between seropositive and seronegative dogs using logistic regression analysis.
Results: Overall seroprevalence of H3N8 CIV in sampled dogs was 49% (95% CI = 46, 51%). Annual seroprevalence ranged from 15% (95% CI = 7, 29%) in 2009 to 62% (95% CI = 56, 68%) in 2007. Region (Northeast, West) and exposure setting (boarding kennels, shelter facilities) were associated with increased odds of seropositivity.
Conclusions: The study results justify the need for continued surveillance of H3N8 CIV in US dogs, and for communal housing facilities to formulate, implement, and evaluate biosecurity protocols to reduce virus transmission risk.

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Effect of Skin lessions on Haematological Picture of some Dogs in Ibadan.

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The study reported the effect of skin diseases on haematological parameters in dogs presented in some veterinary clinics in Ibadan. 44 out of the 80 dogs (different breeds) presented during the period were positive for mange infestation (32 sarcophilic, 10 demodecdic and 2 mangelic) 2 mls of blood was collected from the cephalic vein of dogs that had skin lesion. A comparative hamatological study was done by comparing the results of the dogs with skin

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lesions with apparently healthy dogs without skin lesions. The result obtained shows that Hb, PCV and RBC count of infested dogs (54.5%) fall within the range of 7.8-11.7g/dl (11.9g/dl), 26-34% (30.6%) and 3.42- 4.01 x 10^6_{ul} ($3.4x10^6_{ul}$) respectively which is slightly lower than 11.8 - 14.7g/dl (11.9g/dl), 36-45% (35.9%) and 5.25-6.90 x 10^6_{ul} ($5.33 \times 10^6_{ul}$) recorded for the control. This indicates mild to moderate anaemia in these dogs. The study shows that there is a significant different $P \leq 0.05$ in the PCV, value of dogs with skin lesion when compared with normal dogs. The skin lesions in dogs affect PCV value in dogs.

037
Modeling feral cat population dynamics in Knox County, TN.
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Feral cats (*Felis catus*) are recognized as a problem internationally due to their negative impact on wildlife and potential to spread infectious disease to people and other animals. In addition, many people consider them a nuisance. Others devote significant time and other resources focused on the welfare of feral cats. Trap-neuter-return (TNR) programs are employed in many areas of the country as a more humane method to control feral cat populations and this approach is used on a limited basis in Knox County, Tennessee. Despite the frequent use of TNR as a strategy to reduce or eliminate feral cat populations, its effectiveness remains controversial. The objective of the mathematical model we developed is to predict the impact of selected strategies on the population of feral cats under the current TNR program. The model is based on the assumption that the feral cat population is closed except for births. We tested the impact of spaying females over a five year period with one month time steps and involving five age/spay classes. We tested different TNR parameters to assess how targeting spays seasonally might better address the feral cat problem. Current TNR efforts lack specific targeting and thus possess limited ability to curb population growth. Seasonal targeting of TNR efforts predicted a measurable decline in feral cat population growth over a five year period with the potential for zero population growth and stabilization of the population. The best case scenario resulted in a projected decline of the population within two years if sufficient resources and efforts were applied prior to the breeding season. Cost effectiveness of the strategies was measured by the number of spays conducted each year over the five years. Targeting TNR intervention at adult females during the time of the year prior to mating season (December - February) in areas highly populated by feral colonies may be the most effective in decreasing the feral cat population. These results suggest a more efficacious strategy than non-targeted TNR programs, and provide a humane and cost-effective alternative to trap-euthanasia (TE). Data gaps were also identified.

038
Using logistic regression models to create a scoring system for socialization status of shelter cats during a three day holding period.
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Purpose: Free-roaming cats are often taken into U.S. animal shelters. Their disposition options depend heavily on where the cat appears to fall along the socialization spectrum from truly unsocialized to well-socialized with humans. However, accurately determining socialization status of these cats can be difficult because many cats behave fearfully upon arrival in the novel shelter environment with unfamiliar people. There are currently no validated methods of determining cats' socialization status. Our objective was to develop a valid and reliable method for shelters to distinguish cats who are less socialized with humans from those who are more socialized.
Methods: We studied 253 owned and unowned cats whose socialization status was scored by a survey completed by their caregivers. This 0 to 10 score was dichotomized: less socialized with humans and moderately- to well- socialized with humans. We then studied the cats for 3 days after arrival in a shelter-like setting, using a variety of behavioral and physical measures during 4 assessments in 5 time periods as independent variables. Logistic regression models for each of the 5 time periods were developed with forward stepwise modeling, followed by checking each rejected variable in the model again.
Results: The cats' behavior changed enough over the 3 days that different variables were predictive in different periods; however, cats' ear, body and tail positions, affiliative behavior, aggression and sleeping were significant in multiple time periods. Area under the curve ranged from 0.77 to 0.84. Socialization scores based on each model were developed using the model coefficients. For each model the coefficient for the highest risk category for each variable was summed. An arbitrary maximum score of 50 was divided by this sum to obtain a multiplier. Each coefficient was then multiplied by this number and rounded to the nearest integer. Reference categories received a score of zero.
Conclusions: While models showed modest accuracy, the choice of the best model from which to develop a score for use in a shelter setting was not clear. Work is ongoing to validate these models and refine the scoring methods to improve prediction.

039
Development and implementation of a Shelter Population Index (SPI) to evaluate population trends of cats and dogs in the United States.
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Development of a standardized tool that allows uniform assessment of trends in shelter animal statistics would be valuable in assessing trends of national animal shelter populations. This study was divided into two phases. Phase 1 was a longitudinal survey to test the methodology of a standardized Shelter Population Index (SPI) to track shelter trends of cat and dog populations in U.S. shelters. Phase 2 was a national retrospective study of shelter data with the objective of testing more broadly the methodology developed in Phase 1. Eighteen animal shelters in four U.S. Census Combined Statistical Areas (CSAs) participated in Phase 1. Data collected included the number of animals received by shelters and the numbers adopted, returned to owners, transferred to other agencies, euthanized and died/lost. Estimates were weighted by the human population that the shelters served. Data were used to formulate a hypothetical annual SPI for tracking trends. Phase 1 concluded that data collection for the SPI is feasible and useful. Phase 2 included the collection of data from shelters serving approximately 54% of the U.S. population during 2007 and 2008. Data on approximately 4 million cats and dogs was available for analysis over both years of Phase 2. The SPI can range from -1.000 to +1.000, with a neutral or balancing point of 0.000. An SPI of -1.000 would be the worst-case scenario where all the animals that entered shelters were euthanized or otherwise died. In contrast, an SPI of +1.000 would be the best-case scenario where all animals that entered shelters were adopted, reclaimed by owners or transferred. The neutral point of 0.000 implies that there is a relative

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balance between those animals that have been euthanized and those that find homes. The estimation of the national SPI for cats is -0.204 for 2007 and -0.197 for 2008. The estimation of the national SPI for dogs is better with estimates of +0.269 for 2007 and +0.309 for 2008. Estimates of SPIs by Census Divisions will also be presented. By linking shelter numbers to the human population that the shelters serve, the SPI can focus the issue of a community's cat and dog surplus on the shared responsibilities of the communities.

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040
Investigation of pig mortalities in Maha Sarakham Province, Thailand, October 2010.
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On October 2010, Department of Livestock Development was notified of pig mortalities with clinical signs suggesting an infection by porcine reproductive and respiratory syndrome (PRRS) virus. The event was reported from Laodokmai sub-district, Chuenchom district, Maha Sarakham province. A disease investigation took place between12-15 October 2010. The objectives of the investigation were to determine the magnitude of the outbreak, identify potential factors contributing to disease introduction and transmission and provide recommendations for future prevention and control.
A cross-sectional study was conducted in 7 villages of Laodokmai sub-district. Suspected and confirmed case definitions were formulated for an active case finding. Suspected farm was actively identified using standardized questionnaire interview. Information inquired in the questionnaire included farm demography, clinical signs, epidemiological characteristic of an outbreak and possible risk factors. Serum and semen samples were collected from pigs kept within suspected farms for laboratory confirmation using RT-PCR.
68 out of all 74 small farms in the studied area were visited. A median of number of pigs per farm was 14 (interquartile ranges of 5 to 24). Thirty-seven suspected farms (attack rate=54%) were identified based on the case definition. At the animal level (n=1,139), morbidity, mortality, and case fatality rates were 4 6%, 20%, and 44%, respectively. Piglets were the most affected group (morbidity rate=66%, mortality rate=56%). Utilization of breeding boar from Farm A had odds ratio of 3.95 (95%CI=1.20-13.00), while other factors were not statistically significant at the level of 0.05.The results of RT-PCR showed 3 out of 13 serum samples were positive for PRRS virus.
This outbreak was likely caused by infection of PRRS virus. The outbreak affected primarily small holders and the most affected population was piglets. Removal of persistent carriers, especially breeding boars from farm A, was recommended. Regular serological monitoring of pigs in Laodokmai sub-district, as well as in newly introduced pigs should be considered.
Keywords: PRRS, Investigation, Thailand

041
Adjusting disease freedom confidence for imperfect diagnostic accuracy: A review of the evidence for non-traditional diagnostic specimens tested for PRRSV.
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PURPOSE: Regional Area Control and Elimination projects require cost effective options to monitor for PRRSV including diagnostic specimens and collection tools other than venipuncture. Provided the test performance is known and reproducible when run on alternative samples, the interpretation of disease freedom confidence can account for imperfect performance. This process must be transparent because the results can affect the entire region. This review was undertaken to determine if sufficient evidence was available in published literature to make such adjustments.
METHODS: Structured searches were applied to citation databases. Relevant publications between 2000 and 2010 were reviewed to obtain test sensitivity, specificity, validity, reliability and reproducibility for PRRSV diagnostic tests on the following venipuncture-alternatives: PCR: pooled blood swabs, blood on filter discs (FD), oral fluids (OF), OF on FD, milk, meat transudate and tonsil swabs; ELISA: pooled serum, blood swabs, blood on FD (individual and pooled), OF, milk, and meat transudate.
RESULTS: No option had comparable yet independent peer-reviewed publications from which diagnostic sensitivity, specificity, or reliability distributions could be estimated without substantial assumptions. Tests for OF with PCR and pooled serum with ELISA had sufficient validation detail reported to recommend research focus on reproducibility. Remaining options require continued research on reliability and reproducibility.
CONCLUSIONS: Despite promising developments for monitoring PRRSV, there was insufficient evidence to make recommendations on how the confidence in results from venipuncture-alternatives should be adjusted for test performance. Future publications describing test validation should adhere to recommended standards to ensure that results are maximally useful. Laboratories are encouraged to report test validation results to enable further statistical evaluation of test accuracy.

042
Modeling of porcine reproductive and respiratory syndrome virus infection in a pig herd.
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Purpose: This research used observational data to estimate the basic reproductive number and other parameters of importance for dynamics of PRRS infection in a population. These parameters were then used to build mathematical models of PRRSv spread and control in sows and nursery pigs.
Methods:The observational data included results of PRRS Elisa test on blood samples from 795 newborn piglets sampled longitudinally from 7 swine herds in Ontario. Random effect linear model was used to estimate the duration of maternal immunity and random effect Poisson regression was used to estimate transmission parameter. Based on the parameters estimated from the data and additionally obtained from literature, the production stage-structured susceptible-infectious-resistant (S-I-R) deterministic mathematical model for sows and the age-structured maternally immune-susceptible-infectious-resistant (M-S-I-R) model for nursery pigs were built to include herd demographics and dynamics of PRRS virus infection in 1000-sows herd. Control

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strategies included gilt acclimatization, herd closure, and mass immunization in different combinations.
Results: Under the assumptions used in the mathematical model, herd-closure for at least 40 weeks can eliminate the virus if immunity against PRRSv in sows is assumed to be long-lasting. Mass immunization with 100% efficacy applied simultaneously to all sows could eliminate the infection if a herd was closed for at least 5 weeks after immunization. If the efficacy of mass immunization is below 100%, longer period of herd closure should be planned to control the disease.
Conclusions: Mathematical model is a useful tool to guide the control strategies. This study showed the possibility of PRRS elimination in a herd that is in general agreement with field observations. Duration of immunity and transmission parameter obtained from diagnostic data were critical for the output of the examined control strategies. Using diagnostic data would allow building of stochastic models and further progress in this field.

043
Baseline study in the Niagara region porcine reproductive and respiratory syndrome (PRRS) area regional control and elimination project (ARC&E).
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Purpose: It is challenging to prevent the infection of herds with PRRSv in pig dense areas, and its elimination requires expensive resources. In addition, some of the recently-emerged PRRSv genotypes are more virulent. One strategy that is gaining popularity is a regional approach to PRRSv infection control. The first PRRS area regional control and elimination (PRRS ARC&E) program in Ontario has been initiated in the Niagara peninsula. The primary objective of the program is to implement a regional approach to infection control and to communicate the process both within the elimination area, and more broadly. The objectives of this study are to describe the baseline measurements and to report the findings of risk-factor analysis.
Methods: At the area-level, some inclusion criteria for this project were: moderate pig density, existing natural borders, and the willingness of producers to participate. The project started in the summer of 2010. More than 95% of the producers in the Niagara area are participating, resulting in 76 herds in the program. Twenty two pigs on each site were blood sampled and tested with PRRS ELISA. Additional data obtained included demographic information and geographical coordinates.
Results: Most of the herds (58%) included in the study were finisher barns. 27% of the sites were sow barns. The distribution of other swine premises within a 3 mile radius around each of the sites varied bi-modally between a minimum of 0 (15%) and a maximum of 10 other sites (1.4%). The mode (19%) was 5 other sites. The herd level prevalence in the baseline study was 39%. In the positive sites, only one site was identified as having a single positive animal; all other positive herds had a minimum within-herd prevalence of 50%. Among other factors, the number of PRRSv-positive sites within a one mile radius was identified as a risk factor.
Conclusions: Distribution of within-herd prevalence suggests that a higher level of design prevalence could be justified for surveillance. Risk factor analysis should include membership in swine production networks, and data for such investigations should be included in routine data collection.

044
Association between PRRSV ORF5 genetic distance and differences in space, time, ownership, and animal sources among commercial pig herds.
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Purpose: Open reading frame 5 (ORF5) of the porcine reproductive and respiratory syndrome virus (PRRSV) genome is a variable region of the genome frequently used for diagnostic classification. The objective of this study is to test the association of genetic distance between pairs of PRRSV ORF5 sequences with the distance in space between the herds in which the viruses were collected; the separation in time between when the viruses were collected; and the distances in ownership, animal and semen suppliers between herds.
Methods: Ownership and animal/semen supplier distances were binary measures of either the same or different between two herds. The partial Mantel test was used to test for correlation between genetic, spatial and temporal distances. Multivariable linear regression using simulation to generate P-values was used to test for associations of all variables with the outcome.
Results: Significant correlation was found between genetic distance and space (r=0.10) and time (r=0.03) after accounting for ownership similarity between herds. This correlation was limited to a threshold of < 30 km spatial separation. The slope of association of genetic distance with space and time was positive in herd pairs from different owners but for herds pairs for the same owner the relationship did not hold. Gilt and semen supplier distances were significantly associated with genetic distance in univariable models. After accounting for ownership the associations were overshadowed by the ownership effect.
Conclusions: These findings are an indication that PRRSV is spreading among herds in Ontario, Canada. The spread between herds may be occurring by direct transmission between herds that are < 30 km from one another or via gilt and semen sources that are shared by many herds. The clustering of herds within ownerships is important in PRRSV spread and is important to consider in order to detect spatial patterns of PRRSV.

045
The association between the PCVAD outbreak in Ontario and the positivity of Porcine Reproductive and Respiratory Syndrome virus ELISA and PCR test results.
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Purpose: Animal disease monitoring and surveillance are crucial for ensuring
the health of animals, humans and the environment. Many studies have investigated the utility of monitoring syndromes associated with veterinary laboratory submissions,but limited research has focused on how negative test results from veterinary diagnostic laboratory data can be used to improve our knowledge of disease outbreaks. For example, if a diagnostic laboratory was seeing a disproportionate number of negative
test results could this information be an indication of a novel disease outbreak? The objective of this study was to determine the association between the porcine circovirus-associated disease (PCVAD) outbreak in Ontario 2004-2006 and the weekly probability of Porcine Reproductive and Respiratory Syndrome Virus (PPRSV) enzyme-linked immunosorbent assay (ELISA) positivity and the weekly probability of PRRSV polymerase chain reaction (PCR) test positivity.
Methods: Retrospective data were collected from the Animal Health
Laboratory (AHL) at the University of Guelph, Guelph, Ontario Canada and were comprised of the weekly count of PRRSV ELISA and PRRSV PCR tests ordered by swine practitioners from 2000-2007. The PRRSV ELISA and PRRSV PCR test results were analysed separately in two separate models using logistic regression with the dependent variables: the weekly probability of PRRSV ELISA positivity, and the weekly probability of PRRSV PCR positivity, respectively. The association between PRRSV test positivity and the outbreak of PCVAD was determined after controlling for a PRRS outbreak, season,

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and year.
Results:The weekly probability of PRRSV PCR positivity decreased during the PVCAD outbreak (OR=0.66, *P*<0.01). The weekly probability of PRRSV ELISA positivity was not associated with the PCVAD outbreak.
Conclusions: The results indicate that during the PCVAD outbreak in Ontario from 2004-2006, the probability of PRRSV PCR positivity at the AHL decreased. Tracking the test results of commonly used screening tests has the potential to be a novel data source for the timely identification of disease outbreaks in swine populations.

046
The impact of maternally derived immunity on influenza virus transmission in neonatal pig populations.
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Purpose: One measure to control influenza virus in pig populations and reduce the number of susceptible animals is through vaccination. Vaccination of breeding females is a common practice for influenza virus. Vaccination of sows should not only provide active immunity to the breeding herd, but also passive immunity through colostrum to neonatal pigs. The objective of this study was to assess the role of maternally derived immunity in reducing influenza virus transmission in neonatal pig populations.
Methods: Sows from an influenza virus negative herd were assigned to one of three treatment groups: homologous influenza vaccine (HO), heterologous influenza vaccine (HE), and no vaccine (NV). Sows within the respective treatment groups were vaccinated pre-farrow with the HO and HE killed influenza vaccines. The HO vaccine was created with the H1N1 challenge virus for this study and the HE vaccine was created with an unrelated H1N1 virus. For each of two or three replicates, 10 pigs (3-4 weeks of age) from each of the aforementioned treatment groups were challenged with the HO influenza virus via direct contact with an experimentally infected pig. Nasal swabs were collected daily for 14 days following contact challenge and tested for influenza virus RNA via RT-PCR. Transmission parameters (β), infectious periods, and reproduction ratios (R) were estimated and compared between treatment groups.
Results: All pigs in the HE and HO groups had detectable antibody titers to the respective vaccine antigens and pigs in the NV group were seronegative prior to contact challenge. All contact pigs became infected in the HE and NV groups and the R estimates did not differ significantly at 7.8 and 11.0. One pig in the HO group became infected following contact challenge and the R estimate was significantly lower in this group at 0.84 versus the HE and NV groups.
Conclusions: This study indicates that vaccine induced maternal immunity may be able to reduce influenza virus transmission in population settings; however, the impact will depend on the priming antigen. This study also indicates that influenza virus will spread rapidly in non-immune pig populations.

047
Sensitivity of oral fluids for the detection of influenza virus in young pig populations with and without maternally derived immunity.
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Purpose: Oral fluids have been used successfully for the detection of PRRSv, PCV2, and more recently, influenza virus in pig populations. Due to the short duration of shedding and the potential absence of obvious clinical signs in the presence of maternal immunity, detection of influenza virus can be difficult and often leads to sampling a large number of individual animals. The objective of this study was to assess the sensitivity of oral fluids for the detection of influenza virus in young pig populations with and without maternally derived immunity.
Methods: Nine pens of 10 pigs were each challenged with influenza virus via direct contact with an experimentally infected pig at approximately 3-4 weeks of age. Three pens were seronegative and six pens had varying levels of maternally derived immunity to influenza virus. Nasal swabs and oral fluids were collected for a period of 2 weeks post-exposure to the experimentally infected pig. Nasal swabs were collected from all pigs in each pen and one oral fluid sample was collected from each pen on a daily basis on 102 occasions. All samples were assessed via influenza virus RT-PCR. A pen was considered positive if one nasal swab was positive or if the oral fluid sample was positive. Agreement, sensitivity, and specificity were compared between nasal swab and oral fluid sample results at the pen level.
Results: The overall kappa coefficient for agreement (κ) between oral fluids and nasal swabs was 0.75 (95% CI 0.62-0.87). With nasal swab sampling as the gold standard, the pen sensitivity and specificity of oral fluids was 79% and 100%, respectively. The pen sensitivity of oral fluids increased to 93% when prevalence of infection was greater than 9%.
Conclusions: The kappa coefficient, pen sensitivity, and specificity values in this study were very similar to those estimated in a similar experimental setting with older pigs. This indicates that pig age and maternally derived immunity may not have a large impact on the efficacy of pen based oral fluid sampling. This study provides additional evidence that oral fluid sampling is an effective and sensitive method for the detection of pathogens, including influenza virus, in swine populations.

048
Detection of influenza A virus in aerosols from acutely infected pig populations.
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Influenza A virus transmission in swine occurs mainly by direct nose-to-nose contact and aerosols. Transmission between farms is thought to occur through movement of infected pigs. However, there are reports in which outbreaks of respiratory disease in pig farms have occurred suddenly and are not associated to the introduction of animals which suggest that airborne transmission of influenza between farms may be possible. Information on aerosol transmission of influenza A virus in pigs is scarce.
Purpose: The objective of this study was to detect airborne influenza A virus from acutely infected pig populations in the field.
Methods: Two commercial pig herds acutely infected with influenza A virus were identified. Air samples were collected from inside and outside the barns using a cyclonic air collector. In addition, 15 oral fluid samples were collected from pigs inside the barn. If a population tested positive in the first visit, a second visit was scheduled seven days after the first visit and testing repeated. Air and oral fluid samples were tested for influenza RNA by RRT-PCR.
Results: In farm 1, an H1N2 influenza virus was detected in pigs with acute respiratory signs. All air samples tested RRT-PCR positive for influenza A virus. Virus was isolated from 8 air samples. Virus was also isolated from 11 of 15 oral fluid samples. During the second farm visit, 2 air samples tested positive. All 15 oral fluid samples tested positive. No virus was isolated from these samples. In farm 2, 4 air samples were classified as suspect. All 15 oral fluids tested positive for influenza A. No virus was isolated from these samples.
Conclusions: Our results indicate that acutely infected pig populations can generate viable airborne influenza A virus capable of being exhausted from pig barns and likely disseminated to other farms in the vicinity. Detection of influenza A virus in the field will depend on the course of disease and more studies are needed to further understand the regional airborne spread of influenza A virus.

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Influenza A virus genetic diversity in immune pigs.

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Hemagglutin (HA) protein is the main antigenic protein in influenza viruses. Changes in HA protein drive antigenic drift and immune pressure plays a role in part, in driving those changes. In this study we evaluated the genetic changes in HA of two groups of animals with either acquired immunity or passive immunity. The results from these analyses may help elucidate the mechanisms and causes of increased genetic diversity in influenza A viruses in swine and the possible generation of novel influenza A viruses that may cross species barriers. Virus sequences obtained from two different studies were analyzed to study HA changes in immune animals (with acquired immunity or with passive immunity) that had been subsequently infected with a triple reassortant influenza A virus of swine origin. Overall we observed more genetic changes in the sequences from viruses detected in the pigs with passive immunity than in the pigs with acquired immunity. Both non-synonymous and synonymous changes were observed, meaning that there were genetic changes that resulted in antigenic changes in the HA protein and some that did not result in predicted amino acid changes. In addition, changes appeared to happen in a very short time post infection, within a relative small population of animals, and with pigs of different immune status. Changes in the receptor binding site and other antigenic sites in the HA protein have been described before for influenza A H1 viruses. Some of the changes observed in this study corresponded to changes previously described in the literature. We are currently analyzing the substitutions observed in this study in the context to help elucidate the mechanisms and causes of increased genetic diversity in influenza A viruses in swine and the possible generation of novel influenza A viruses that may cross species barriers.

050

Estimating hiding behavior for lameness in sows.

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Purpose: The purpose of this study was to estimate the extent of hidden lameness in sows. It is common in many species that lameness is hidden when animals feel threatened. This may also apply when lameness detection tests are conducted. This reaction can present difficulties for diagnosticians in estimating the prevalence of lameness in sows.
Methods: 48 sows were observed twice - once for their behavior during a feeding session, considered gold stand for lameness detection in this study, and another while they were being moved though the walkway to farrowing crates. These observations were performed by the same person but blinded to ID. Based on their behavior, they were recorded for lameness (0 =non-lame, 1=lame). We bootstrapped the distribution of the differences in lameness scores with 10000 iterations. The possible different outcomes of the algorithm were 0 (no change), +1 and -1. The difference of +1 (measured) means a sow was categorized as lame in the first observation and was categorized as non-lame in the second observation, and vice versa for -1 (error). The hidden lameness was estimated from the model $\mu hiding = \mu measured - \mu error$.
Results: Findings from the analysis indicate that the proportion of non-lame sows in the second observation, which were judged non-lame in the first observation, was 96%. The proportion of sows which were found to be lame only in the second observation was 4%. The proportion for sows which were from the lame group in the first observation but turned out to be non-lame in the second observation was 37.5%, and that those that stayed lame in both observations was 62.5%. The proportion test showed a significant difference between the two methods of detection ($\chi 2 = 15.844$, p-value < 0.0005). The proportion of the 'measured' (1), 'no change' (0) and 'error' (-1) were 66.0% (95 PCTCI [64.1%, 67.0%]), 77.4% (95% PCTCI[76.0%, 80.1]), and 33.1% (95%PCTCI[32.3%, 35.2]) respectively. Therefore, the hiding proportion estimated was 32.9% (95 PCTCI[29.4%, 34.1%]).
Conclusions: Hiding behavior can be a significant source of error in measuring lameness, and the potential decrease in likelihood of diagnosis must be considered.

051

The effect of lameness and other morbidity causes on average daily gain in feedlot cattle.

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Purpose: Lameness is detrimental to the health and well-being of feedlot cattle and their growth performance. Causes of feedlot lameness include toe abscesses, footrot, injury, joint infection, and laminitis. The objective of this study was to quantify the effect of feedlot lameness on average daily gain (ADG).
Methods: Animal health data from 14,852 feedlot steers was obtained from the Meat Animal Research Center (MARC), Clay Center, NE. Disease variables were categorized as follows: 1) no disease; 2) disease less than 60 d; or 3) disease 60 d or later, in days of age for calves before entry into the feedlot for pre-weaning diseases, or days on feed until the animal was treated in the feedlot for feedlot diseases. Multivariable general linear mixed modeling was used to test effects of disease on ADG with year as a random effect variable.
Results: Variables significantly explaining ADG were lameness, respiratory disease, bloat, digestive system disease in the feedlot, and respiratory disease and pinkeye prior to weaning, days on feed, and the number of days between taking the last weight and when cattle left the feedlot. The overall ADG for cattle in the feedlot was 1.34 kg/d. Compared to cattle without the disease, cattle treated for lameness, bloat, or digestive system disease 60 d or later in the feeding period had a decrease in ADG of 0.019 kg/d ($p=0.046$), 0.062 kg/d ($p=0.0176$), and 0.068 kg/d ($p=0.0052$), respectively. Animals treated for respiratory disease in the feeding period had a decrease in ADG by 0.022 kg/d ($p<0.0001$). In contrast, steers treated for respiratory disease or pinkeye late in the pre-weaning period had 0.030 kg/d ($p<0.001$) and 0.022 ($p=0.0026$) kg/d, greater ADG in the feedlot, respectively.
Conclusions: Lameness in steers had a significant and meaningful negative effect on ADG. Rate of growth was affected not just if lameness or other morbidity causes occurred, but when the event occurred.

052

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

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Purpose: *Salmonella enterica* has been commonly recognized as a cause of nosocomial infections as well as zoonotic infections in veterinary teaching hospitals (VTHs). Additionally, there have been multiple reports of VTHs restricting patient admissions to mitigate epidemics of nosocomial *S. enterica* infections. The objective of this study was to determine risk factors associated with environmental contamination of a veterinary teaching hospital with *S. enterica*.
Methods: Environmental surveillance samples were collected from February 2003 through June 2011, using a commercially available electrostatic wipe, as part of the ongoing infection control program. Sampling sites included both floor and hand contact surfaces throughout the VTH. Risk factors evaluated included hospital case load, hospital use areas, average duration of hospitalization, presence of culture positive inpatients and season. Data on risk factors

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of interest were collected retrospectively from the VTH medical records database. Multivariable Poisson regression was used to evaluate associations between hospital risk factors and veterinary hospital environmental contamination with *S. enterica*.
Results: During the study period, approximately 53 samples were collected monthly, for a total of 5337 environmental samples. Of the samples collected, a total of 7.9% (n=423) were culture positive for *S. enterica* using standard culture techniques.
Conclusions: Risk factors identified in this study will allow for the refinement of existing infection control programs as well as provide guidance to those in program development. A better understanding of the risk factors associated with environmental contamination will allow for more practical evidence based preventive measures to be implemented in veterinary hospitals experiencing epidemics of nosocomial infections with *S. enterica*.

053
Salmonella shedding in hospitalized horses with signs of colic, with or without diarrhea.
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Purpose: Several epidemiologic studies have showed that the frequency of hospitalized horses shedding *Salmonella* during hospitalization is higher among horses with signs of colic without diarrhea than in horses with diarrhea. This subpopulation of horses without diarrhea presents a threat to hospitals because *Salmonella* shedding is often detected too late, after environmental contamination and/or disease transmission has already occurred. The objective of this study was to investigate the relationships between clinical signs, hematology and serum chemistry parameters, and clinical procedures (before admission, at admission, and during hospitalization) and *Salmonella* shedding in hospitalized horses with signs of colic, with or without diarrhea.
Methods: The study population included adult horses with signs of colic admitted to the UF Large Animal Hospital during 2007-2010. A matched case-control study approach was used to compare the frequency of investigated exposure factors between case and control horses matched by year of admission. Conditional logistic regression analysis was used to model the odds of being a case as a function of investigated exposure factors. The analysis included 14 case horses with diarrhea, 46 case horses without diarrhea, and 73 control horses.
Results: Results revealed that low serum sodium at admission was associated with *Salmonella* shedding in horses with diarrhea (OR = 15.70; 95% CI = 2.36, 104.23). In addition, high plasma protein concentration at admission (OR = 4.95; 95% CI = 1.33, 18.46) and low plasma protein concentration during hospitalization (OR = 3.62; 95% CI = 0.92, 14.24) were associated with *Salmonella* shedding in horses without diarrhea.

054
Management practices associated with *Salmonella* or antimicrobial resistant *Salmonella* on United States dairy herds.
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Salmonella is the leading cause of foodborne related deaths and hospitalizations within the United States. Infections caused by antimicrobial resistant (AMR) strains are associated with higher hospital costs and higher case fatality rates. Data from the 2007 National Animal Health Monitoring System study of U.S. dairy herds were used to determine the association of modifiable management practices with the recovery of *Salmonella* and AMR *Salmonella.* Individual adult cow fecal samples and/or composite environmental samples were collected from 265 dairy herds in 17 states. Samples were cultured for *Salmonella*, and the minimum inhibitory concentration (MIC) was determined for 15 antimicrobials. Resistant isolates were those in which the MIC exceeded the established breakpoint for any antimicrobial. Herds were classified as *Salmonella* positive if at least one isolate was recovered, and AMR positive if at least one resistant isolate was recovered. Questionnaires regarding management practices were administered to herd operators, and a subset of potential risk factors was selected based on subject knowledge and prior research. Questions on antimicrobial usage were collapsed to denote either use or lack of use of each antimicrobial class within each animal type (cow, heifer, or calf). Univariate and multivariate logistic regression were used to determine which practices were significantly ($p < 0.05$) associated with each herd classification. A total of 124 and 26 herds were classified as *Salmonella* positive and AMR positive, respectively. Factors significantly associated with *Salmonella* positive herds included using sprinklers or misters for heat abatement (OR = 2.8; CI: 1.6-4.9), feeding anionic salts to close-up cows (OR = 2.0; CI: 1.1-3.5), lack of use of a broadcast or solid spreader (OR = 3.8; CI: 1.6-9.0), and feeding ionophores to cows (OR = 2.1; CI: 1.2 - 3.7). Herds with at least one resistant isolate were more likely to have used composted/dried manure for bedding relative to herds with only susceptible isolates (OR = 3.6; CI: 1.2 - 11). These results can be useful to focus additional research aimed at decreasing the prevalence of *Salmonella* and AMR *Salmonella* on U.S. dairy herds.

055
Phenotypic and genotypic characterization of methicillin-resistant *Staphylococcus pseudintermedius* in dogs, cats, and horses at a veterinary teaching hospital.
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Purpose: Prevalence of methicillin-resistant *Staphylococcus pseudintermedius* (MRSP) in veterinary clinics ranges from 0-42%. The passive surveillance program of The Ohio State University Veterinary Medical Center and Infectious Diseases Molecular Epidemiology Laboratory aims to improve infection control policy and patient care. Objectives are to determine MRSP frequency and relationship between phenotypic and genotypic oxacillin resistance, characterize SCC*mec* types, and determine the level of clonality among MRSP isolates.
Methods: Samples were submitted to the Clinical Microbiology Laboratory (n = 4,684). Antimicrobial susceptibility was determined by Kirby-Bauer disc diffusion and Sensititre. Multiplex PCR was used to identify the *mecA* gene and SCC*mec* types. Pulsed-field gel electrophoresis was used to determine clonal relatedness.
Results: *S. pseudintermedius* (n = 886) was cultured from canine (n = 861), equine (n = 5), and feline patients (n = 20); 816 samples were available for genotyping. Phenotypic (n = 310) or genotypic (n = 391) MRSP comprised 6.6-8.3% of bacterial isolates. Most samples were from skin (44%) in dogs and urinary system (35%) in cats, with no predominate site in horses. Phenotypic oxacillin resistance was 36% in dogs, 0% in horses, and 32% in cats, while the resistance genotype was seen in 48% of dogs, 20% of horses and 53% of cats. Phenotypic and genotypic resistance agreed in 94% of canine and 100% of feline samples. Most canine (40%) and three feline isolates (30%) were SCC*mec* Type V or VII, while 40% of dog and 30% of cat samples were non-typeable. There was a high level of clonality among organisms from unrelated canine patients at different times within the same service.
Conclusions: Overall MRSP frequency was low. There was a higher level of genotypic than phenotypic resistance but good agreement among phenotypic and genotypic resistance in dogs and cats. A high number of dog and cat isolates were non-typeable. The level of clonality may indicate persistent or recurrent environmental contamination or reflect a feature of MRSP in the overall canine population.

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Phenotypic and genotypic characterization of Methicillin-resistant *Staphylococcus aureus* in dogs, cats, and horses at a veterinary teaching hospital from 2007 to 2010.

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Purpose: Prevalence of methicillin-resistant *Staphylococcus aureus* (MRSA) in veterinary hospitals is variable. The Ohio State University Veterinary Medical Center and Infectious Diseases Molecular Epidemiology Laboratory established a passive surveillance system to improve biosecurity and infection control procedures and veterinary patient care and overall public health. Aims include determining the frequency of MRSA and the relationship between phenotypic and genotypic oxacillin resistance, characterizing SCC*mec* types, and determining the level of clonality among *S. aureus* isolates.
Methods: Patient samples were submitted to the Clinical Microbiology Laboratory (n = 4,684). Antimicrobial susceptibility was determined by Kirby-Bauer disc diffusion and Sensititre. Multiplex PCR was used to identify the *mecA* gene and SCC*mec* types. Pulsed-field gel electrophoresis was used to assess clonality.
Results: *S. aureus* (n = 132) was collected from canine (n = 83), equine (n = 33), and feline patients (n = 20); 124 samples were available for genotypic analysis. Phenotypic (n = 62) and genotypic (n = 56) MRSA comprised 1.2-1.3% of bacterial isolates. Samples were primarily from musculoskeletal tissue (35%) or soft tissue injury/infection (30%) in dogs, soft tissue injury/infection (70%) in horses, and skin (45%) in cats. Phenotypic oxacillin resistance was 53% in dogs, 49% in horses, and 26% cats. Phenotype and genotype agreed in 95% of dogs, 93% of horses, and 100% of cats, while 52% of dogs, while 44% of horses and 18% of cats were resistant by genotype. Canine and feline isolates were mainly SCC*mec* Type II, while equine samples were SCC*mec* type IV. PFGE revealed a high level of clonality among isolates from apparently unrelated canine patients during different time periods within the same department at the VMC.
Conclusions: Frequency of MRSA was low. Canine and equine isolates had similar origin and level of oxacillin resistance but differed in SCC*mec* types. Oxacillin resistance in the cat was lower than in dogs and horses. It is unclear if the level of clonality indicates persistent or recurrent environmental contamination or reflects a feature of MRSA in the overall canine population.

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Using agent-based modeling of the village poultry sector in Thailand to identify opportunities for influenza transmission and potential interventions for disease control.

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Background: Highly pathogenic avian influenza (HPAI) virus H5N1 has impacted poultry production, human and avian health in Southeast Asia and beyond. Ducks are considered a bridging species for influenza virus transmission between wild waterfowl and gallinaceous poultry, and free-grazing duck flocks have been identified as a risk factor for HPAI H5N1 poultry outbreaks in Thailand. Free-grazing duck farmers move flocks of 1,000 or more ducks among post-harvest rice fields, where they feed on residual rice, insects and snails. While flocks can move > 50 km between provinces, most movements are shorter, occurring on the village or subdistrict level. Little is known about the dynamics of HPAI transmission in this local poultry sector, which includes free-grazing ducks, backyard flocks, farmed poultry, egg and poultry traders. Purpose: We aim to describe interactions within the Thai village-level poultry sector and develop an agent-based model of this poultry network. Using the model, we will identify interactions that could result in between-flock influenza virus transmission. By evaluating the impact of movement and trade within the model, we aim to identify interventions that will have an impact on the risk of transmission while minimizing negative socioeconomic implications for flock owners. Methods: We conducted semi-structured interviews with owners of backyard and farmed chickens, free-grazing ducks, fighting cocks, as well as egg and live poultry traders to determine the frequency and character of poultry-related contacts, including transport, sale and purchase of birds and eggs. Poultry owners were identified with a local official from the Department of Livestock Development, and traders were identified by snowball sampling after poultry owner interviews. This data has been used to develop an agent-based model of the village poultry network. Such models combine theory and computation to produce a computerized artificial society, allowing for the observation of how micro-level decisions can affect macro-level patterns. The model was built with NetLogo software (Northwestern Univ., IL). Data collection occurred in March, April and September of 2011.

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Hierarchical Baian modeling of heteroskedasticity in average daily weight gain of feedlot cattle.

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Average daily gain (ADG) is a key performance outcome in feedlot cattle. Just as relevant as overall mean ADG is the uniformity and consistency of responses around the mean. Objectives of our study were to assess the variance of ADG as a measure of uniform performance and to investigate cohort- and feedlot-level demographic factors as potential sources of heteroskedasticity, while accounting for the hierarchical data structure. The dataset contained 24,050 cattle cohorts from 25 U.S. feedlots during 2005 and 2006. Inference was based on a hierarchical Baian model implemented with Markov Chain Monte Carlo, whereby cohorts were modeled at the residual level and feedlot-years were modeled as clustering random effects. Forward model selection based on Deviance Information Criterion was used to screen potentially important explanatory variables for heteroskedasticity. Number of animals in the cohort was the most influential fixed-effect factor on cohort-level variance, with uniformity of ADG increasing with cohort size. Cohorts had most variable ADG in summer (July to September) and most uniform (least variable) ADG in winter (January to March). Cohorts with fewer days on feed (DOF) had more variable ADG relative to those with more DOF. Arrival weight also contributed significantly to heteroskedasticity in ADG, though effects were smaller in magnitude relative to other cohort-level factors. The random effect of feedlot-year clusters was by far the greatest contributor to ADG heteroskedasticity. These results grant further investigation of sources of heteroskedasticity in feedlot performance with the ultimate goal of targeting uniform and consistent beef production.

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Culling decisions based on microbiological and serological test results for the control of Johne's disease in beef cow-calf operations.

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Purpose: A decision tree model was developed to identify the most economically beneficial decision under the scenarios of no testing, ELISA only testing, or bacterial culture of feces only testing in a truly infected beef cow-calf herd at different Johne's disease prevalence levels.
Methods: Information inputs concerning weaning weight of calves based on the ELISA and fecal culture test status of dams were estimated from Johne's Disease Demonstration Herd Project (JDDHP) data. Lowered reproductive performance, losses due to premature culling and replacement, and the costs associated with testing were obtained from surveys of cow-calf producers. The model assessed different permutations of test (test or not test), test type (fecal culture or ELISA), true status of the animal (true positive or negative and false positive and negative), culling decision (retain or cull) and reproductive performance (cow calves or remains open). Value of each node was estimated using information on calf weaning weights from JDDHP data, reproductive performance of cows from a producer survey and other costs based on published parameters and prevailing prices of cull cows and weaned calves. The estimated value of each node of the tree was evaluated for the best management suggestion over different levels of true prevalence.
Results: Testing is cost-effective when the true prevalence is 7% or greater. Compared to fecal culture, testing with ELISA provides a greater return on investment for a single test and cull situation but costs incurred over a multi-year program should be evaluated before making a decision.
Conclusions: Optimal management suggestions for testing and culling decisions in a cow-calf herd can be made using herd specific values in a decision tree framework. A multi-year simulation model is necessary for more accurate management suggestions.

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

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Purpose: The objectives of this study were to 1) estimate the prevalence of antimicrobial resistance in the study population and 2) to investigate the associations between exposures to antimicrobial drugs and antimicrobial resistance in fecal non-type specific *E. coli* (NTSEC) recovered from individual feedlot cattle.
Methods: Two-stage random sampling was used to identify cattle for enrollment at 4 western Canadian feedlots. A fecal sample was collected per rectum from each individual at arrival and at a second sampling point around mid-feeding period when cattle were rehandled as part of standard production practices. From samples collected at this second time point, a total of 2726 NTSEC isolates were tested for susceptibility to antimicrobial drugs by disk diffusion and/or broth microdilution. Parenteral and in-feed exposures to antimicrobial drugs were recorded for each individual enrolled in the study as well as for other animals housed in the same pen. The least square means estimates and 95% confidence intervals for the prevalence of resistance at each time point were modeled using logistic regression. Multivariable logistic regression modeling was used to investigate associations between antimicrobial resistance and exposure to antimicrobial drugs. Regression models were adjusted for clustering of observations among isolates, individuals, and pens.
Results: Resistance was most commonly detected in arrival samples for sulfisoxazole (6.4%; 95%CI: 5.2-7.9), streptomycin (6.5%; 95%CI: 5.3-8.0) and tetracycline (19.5%; 95%CI: 17.3-21.9). At the second sampling point (33-202 days on feed), resistance prevalence was 22.8% (95%CI: 20.8-25.0) for sulfisoxazole, 25.5% (95%CI: 23.3-27.8) for streptomycin, and 75.2% (95%CI: 73.1-77.3) for tetracycline. Regression modeling identified an association between exposures to tetracyclines with antimicrobial resistance to tetracycline at the second time point. Exposures to other classes of drug were not associated with increased resistance.
Conclusions: Antimicrobial drugs used in this population of feedlot cattle were not hugely influential of antimicrobial resistance at mid-feeding period.

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Effect of intervention strategies on ceftiofur resistance determinant (blaCMY-2 gene) and its relationship with TetA and TetB genes in 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 and its relationship with the TetA and TetB genes as determined by quantifying the target genes in fecal community DNA.
Methods: A controlled field trial was conducted on 176 steers (interim data presented here are from 88 steers for blaCMY-2 and TetA genes). 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 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 blaCMY-2, TetA, and TetB gene copies/µl of DNA were determined using quantitative real time PCR. The relationship between the quantities of blaCMY-2, TetA genes (ln copies/µl) and explanatory variables (CTC and mixing (MIX) in a full factorial design interacting with period (DAY)) was assessed using multivariate model.
Results: A full factorial model including all main effects, 2-way and 3-way interaction terms was highly significant ($P<0.0001$). CTC had a strong effect of increasing both blaCMY-2 and TetA gene copies consistently across other factors ($P<0.0001$). Mixing had a period-specific effect of decreasing the gene copies inconsistently across other factors.
Conclusions: The preliminary data shows that CTC favors rapid expansion of both blaCMY-2 and TetA genes. Mixing has a significant, though inconsistent, sparing effect on both the target genes. A strong positive correlation was seen between blaCMY-2and TetA genes ($P<0.0001$). Results (blaCMY-2, TetA, and TetB gene copies/µl of DNA) from the full dataset (n=176 steers) will be presented and compared with the concurrent phenotypic and genotypic analysis of E. coli from same fecal samples.

EPIDEMIOLOGY AND ANIMAL HEALTH ECONOMICS

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Genotypic characterization and comparison of tetracycline-resistant *Escherichia coli* isolates arising from humans and swine in a vertically integrated agri-food system.

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Purpose: The objective of this study was to determine the prevalence of 6 *tet* genes: *tet*(A), *tet*(B), *tet*(C), *tet*(G), *tet*(M) and *tet*(O) among phenotypically tetracycline-resistant *E. coli* isolates arising from fecal material collected from integrated human and swine communities.
Methods: Multiple *E. coli* isolates were collected and phenotypically characterized for antimicrobial susceptibility as part of a previously reported study. In this study, 60 swine and 102 human isolates were re-analyzed representing nine distinct tetracycline-resistant phenotypes (ranging from singly resistant to multiple resistant isolates) of which four were common to both hosts while the others were unique to either swine (2) or human (3) isolates. Previously frozen bacteria were thawed and cultured on blood agar at 37°C for 24 hours. Genomic DNA was extracted by boiling bacterial colonies in RNAse free water for 10 minutes. Detection of *tet* genes was by PCR followed by capillary electrophoresis in the QIAxcel system (Qiagen). Fisher's exact test was used to compare the proportion of *E. coli* isolates that carried specific genes across phenotypic profiles and by host species.
Results: Out of the 6 genes tested in our isolates, only *tet*(A), *tet*(B) and *tet*(M) were detected. Overall, 39%, 43% and 16% of the *E. coli* isolates carried *tet*(A), *tet*(B) or *tet*(M) respectively. Six isolates (3.7%) were positive for both *tet*(A) and *tet*(B). Proportion of *E. coli* isolates carrying *tet*(M) was significantly higher in swine isolates than human isolates for the common resistance phenotypes (P=0.036). All three genes detected from human isolates were significantly associated with phenotypic profile (P<0.05). However among swine isolates only *tet*(A) was significantly associated with phenotypic profile (P=0.002). All 10 isolates tested from the resistance profile with ceftiofur were also positive for *tet*(A). No human *E. coli* isolates in our samples were positive for ceftiofur resistance (a phenotype
unique to swine in our study).
Conclusions: Our results indicated that *tet*(A) and *tet*(B) were the predominant genes and the association of *tet* genes with specific tetracycline resistance phenotypes varied by host species.

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Epidemiologists: we're not the same as statisticians.

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Traditionally, epidemiologists have studied the incidence, distribution, and possible control of diseases and other factors relating to health. Consequently, the core skill taught in epidemiology graduate programs is how to understand disease causation. Key concepts taught in epidemiology programs focus on understanding sources of bias in scientific studies, observational study design, describing observational data and understanding the inference reached from the results. Another key area for veterinary epidemiologists is the design and assessment of disease surveillance programs. In a multidisciplinary project, an epidemiologist brings unique expertise to the design stage of the process, particularly when the study is observational or a controlled trial. An epidemiologist should be able to refine the design to maximize the likelihood that the project addresses the study question and reduced biases to the extent possible. If the individual has expertise in the needed statistical method the epidemiologist may also be able to conduct the statistical analysis. This presentation will discuss the skill set that epidemiologists need to succeed in an academic environment, the challenges epidemiologists face to establish sustainable research groups and, the skills, other than the ability to do a t-test, that epidemiologists bring to multidisciplinary collaborations. Further, we will discuss if the skill set needed by the academic epidemiologist differs from that needed by the applied epidemiologist, and discuss if graduate programs are addressing that need.

064

Understanding geographic epidemiology: the geographic epidemiologic trillium.

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Purpose: Spatial or *geographic epidemiology* is a specific branch of *modern epidemiology* and thus follows the same principles even though specialized methods are used. Unfortunately key terms and concepts such as disease cluster and disease clustering are not clearly defined in the literature. It is therefore of utmost importance to gain a common principle understanding of such concepts for communicating research results.
Methods: The ultimate goal of epidemiologic research is the investigation of causal relations in order to control and prevent adverse health outcomes. For the study of causal relations several models of cause have been proposed in the literature most notably the *line model*, the *epidemiologic triangle* and the *web of causation* (Bhopal, R., 2008, Concepts of Epidemiology, 2nd edn. Oxford University Press, Oxford).
Results: Here the relation of these models is discussed within the context of *geographic epidemiology*. Specifically the analogy of the *geographic epidemiologic trillium* is proposed as a means to better understand the basic concepts of *disease cluster*, *disease clustering*, *spatial trend* / *high risk area*, and *geographic correlations*. This analogy provides a new and unified perspective of geographic epidemiologic research concepts and methods. The trillium analogy also makes reference to the *web of causation* and *Chinese boxes* of the *eco-epidemiologic era*.
Conclusions: The trillium analogy provides clear notions for the spatial patters of trend, cluster and clustering.

065

Methodological comparisons for antimicrobial resistance surveillance in feedlot cattle.

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Purpose: The objective of this study was to compare methodological options that might be considered when designing a surveillance program for antimicrobial resistance in feedlot cattle to determine if they were systematically associated with detected differences in resistance in *Escherichia coli* and *Mannheimia haemolytica*.
Methods: These assessments included comparison of resistance in *M. haemolytica* vs. *E. coli* when collected from the same individuals, collection of fecal samples per rectum vs. using pooled samples collected from the ground, comparison of the likelihood of detecting resistance in *E. coli* collected from *M. haemolytica*-positive vs. *M. haemolytica*-negative cattle, and broth microdilution vs. disk diffusion for detection of resistance. Cattle were housed in large commercial feedlots. Susceptibility results for these comparisons were analyzed by logistic regression using generalized estimating equations to control for correlation created by population structure, and by conditional logistic regression.
Results: Resistance was more likely to be detected in *E. coli* than in *M. haemolytica* isolated from the same animals. There were no detectable differences in the likelihood of detecting resistance between *E. coli* isolates recovered from individual fecal samples vs. isolates recovered from pooled (pen-floor) samples at mid-feeding period or between *E. coli* recovered from *M. haemolytica*-positive cattle compared to *E. coli* recovered from individuals negative

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for *M. haemolytica*. Differences were found in the likelihood of detecting resistance between disk diffusion and broth microdilution in *E. coli* and *M. haemolytica* recovered from the same animal.
Conclusions: *E. coli* cannot be used to predict the resistance in respiratory pathogens. Both sampling from pooled - pen floor samples or culturing only *E. coli* from individuals positive for *M. haemolytica* are options to aid convenience without affecting estimates of resistance. When selecting an antimicrobial susceptibility testing method, benefits and drawbacks need to be considered as it appears that there might be differences in the prevalence of resistance detected.

066
Multi-drug resistance in Ontario swine *Streptococcus suis*, *Escherichia coli* K88, and *Pasteurella multocida* isolates (1998 - 2010).
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As part of a larger project designed to evaluate antimicrobial resistance in Ontario swine pathogens, trends in multi-drug resistance in *Escherichia coli* K88, *Streptococcus suis*, and *Pasteurella multocida* were assessed. Passive surveillance data were obtained from clinical submissions to the Animal Health Laboratory between January 1998 and October 2010. Poisson models were used to determine how the proportion of resistance has changed over time relative to the number of drugs tested. The dependent variable was the number of antimicrobials to which the isolate was resistant, and the offset was the number of antimicrobial drugs tested for susceptibility. For all three pathogens, year was a significant variable; however, the trends over time were quite distinct among the three pathogens. The degree of resistance has been declining for *E. coli* K88, increasing for *S. suis*, and was variable for *P. multocida*. Differences in the degree of resistance among the three pathogens may be due to a number of factors. As the data are clinical submissions rather than random samples, different trends in resistance among these pathogens may result from different trends in drug use at different levels of the industry, differences in the management of specific bacterial infections, and/or the management of viral infections that may be associated with a particular bacterial pathogen. Triangulation with other data sources may be required to confirm the factors driving differing trends in multi-drug resistance from swine pathogens.

067
Effect of bovine Corona virus shedding and seropositivity on the risk for BRD in calves transitioning from the farm of origin to the feedlot.
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Purpose:Several studies have previously been conducted to evaluate the association between BCV and BRD in feedlot cattle. The collective results of these studies have not been conclusive and arrive at inconsistent and often significantly different results. The aim of the present case- cohort study was to determine if exposure to bovine corona virus prior to feedlot entry is associated with subsequent BRD occurrence. The null hypothesis tested was that calves shedding or seropositive for bovine corona virus at pre-conditioning have the same BRD occurrence (based on treatment rate) as calves without evidence of bovine corona virus shedding/seropositivity.
Methods:A total of 699 calves were enrolled in the study representing 8 individual farms of origin and five feedlots. Each calf had nasal swabs and fecal samples collected at three time points (farm of origin during preconditioning, arrival at feedyard, and 1 month after arrival at feedyard) for PCR detection of BCV. Furthermore, a serum sample was collected from each calf at each time-point for BCV viral neutralization and BVDV Type I and II viral neutralization.
Results:BCV circulation and shedding was present on all eight farms at all times points studied. A 100% seropositive rate prevented determining risk factors based on serology, although levels of exposure were not a risk factor.
Conclusions:When viral shedding status by fecal, nasal or combined status was evaluated there were no significant associations of shedding with BRD status. As has been observed in other sero-epidemiology studies, titers to BVDV at arrival were associated with increased risk of disease even in a vaccinated population. Although the serologic results of this study are similar to previously reported by other studies the unique feature of this study is the inclusion of the PCR testing and shedding data for BCV at several times during the calf transition to the feedlot.

068
Q-fever in small ruminants in Indiana.
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Purpose: *Coxiella burnetii* is the causative agent of Q-fever, a zoonotic disease of ruminants. Infected animals may exhibit reproductive problems such as abortion, although asymptomatic cases are common. The bacterium is shed at parturition, and continued shedding can occur in milk, urine and feces. Transmission can be through inhalation or ingestion. In humans, about half of those infected will develop disease including flu-like illnesses, and a smaller percentage will go on to develop pneumonia or hepatitis. A very small percentage may become chronically infected. While few human and animal cases have been identified in Indiana, the prevalence of this disease is unknown.
Methods: In order to estimate the prevalence of Q-fever in Indiana small ruminants, two approaches were taken. In the first study, blood samples were collected from 417 goats and 295 sheep at a slaughterhouse in southern Indiana from Oct. -Dec., 2010. All sheep and goats regardless of age or breed were included if they had an Indiana ear tag. Serum was separated and tested by ELISA for the presence of anti-*Coxiella burnetii* antibodies. In the second study, milk samples were collected from 317 dairy goats from 31 farms during June 2011 and a questionnaire was administered. Real time PCR was used to detect shedding.
Results: ELISA testing identified one goat (0.2%) with antibodies and no sheep (0%). In the second study, two of 31 farms (6%) had goats shedding Q-fever. A total of 10 goats (3.2%) were positive (4/5 and 6/37 goats on each farm, respectively). Goats less than two years of age were more likely to be shedding than older goats ($p< 0.001$) with eight of the ten positive goats being less than two. About half of the goat owners interviewed had previously heard of Q-fever. Eighty percent of farmers stated that the goat milk was used for raw milk or cheese consumption, a known risk factor for zoonotic transmission.
Conclusions: Overall, this study found that Q-fever is present in Indiana goats. Additional studies are needed to further characterize the distribution and impact of this bacterium in animals and people in Indiana. Goat owners should be more informed about Q-fever and ways to prevent herd infection and zoonotic transmission.

EPIDEMIOLOGY AND ANIMAL HEALTH ECONOMICS

069

Seroprevalence of brucellosis and its association with other reproductive diseases in buffaloes in Bangladesh.

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An experimental survey was conducted to determine the seroprevalence of brucellosis in buffaloes in Bagerhat and Mymensingh region of Bangladesh from December 2010 to May 2011. A total of 70 sera samples were collected from Bagerhat and Mymensingh region. Among buffaloes sera sample , 24 buffaloes sera samples were collected from Bagerhat and 46 buffaloes sera samples were collected from Mymensingh district. Questionnaire based data on risk factors were collected in buffaloes. A total of 70 buffaloes sera samples were screened by Rose Bengal Test (RBT). Positive, doubtful and negative samples were further confirmed with Indirect Enzyme Linked Immunosorbent Assay (i-ELISA). The overall serological prevalence derived from the samples was 4.28%**.** In conclusion, it is observed that, a higher prevalence of *Brucella* was found in female 6.82% by RBT and 4.55% by i-ELISA than male, aged animal than young and animal with reproductive disorder than animal without reproductive disorder and a history of previous abortion was associated with the highest prevalence of brucellosis (33.33%) than that of other reproductive diseases The result showed that female animal has more possibilities to infection of brucellosis. We studied seroprevalence of brucellosis in buffaloes in two region of Bangladesh. So, Some future recommendations to address this disease may be-regular sero-monitoring of the buffaloes sample need to be carried out, positive reactors must not be used in breeding purpose, people involved in buffaloes husbandry and meat handlers should be cautious not to do anything without gloves if there is any suspicion of brucellosis, programs to raise awareness about this disease should be conducted. This is the first report about seroprevalence of brucellosis and its association with other reproductive diseases in buffaloes in Bangladesh.

070

Prevalence of *Toxoplasma gondii* in market age lambs in the United States.

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Purpose: The objective of this study is to estimate the seroprevalence of *T. gondii* in market age lambs on U.S. sheep operations as part of the NAHMS Sheep 2011 study.
Methods: Blood was collected from 2,125 market age lambs (3-12 months of age) on 200 operations in the 22 largest sheep producing States. Sera were tested for antibodies to *T. gondii* using the modified agglutination test (MAT).
Results: Of lambs tested, 1,781 (16.2%) tested positive with a titer of 25 or above. Lambs in the west had the greatest seroprevalence (25.0%) followed by the east (13.5%) and central U.S. (8.3%). The seroprevalence was 18.0% in operations with fewer than 500 ewes and 8.1% in operations with 500 or more ewes. By flock type, seroprevalence was 19.3% in operations that managed their sheep on pasture compared with 7.5% in open range flocks.
Of the 200 operations, 55.0% (110) had at least one seropositive sample. By flock size, operation-level prevalence was greatest among operations with 100-499 ewes (65.8%), followed by operations with fewer than 100 ewes (51.1%) and 500 or more ewes (36.7%). Operation-level prevalence was greatest among operations in the west (72.4%) followed by the east (48.7%) and central U.S. (37.7%).
Conclusions: Dubey et al. 2007 found a seroprevalence of 27.1% in lambs from MD, VA, and WV at slaughter, while seroprevalence for lambs in the east in this study was 13.5%. Studies have found Toxoplasma titers to increase with age, which may in part account for this difference along with the size, type and location of the flocks of origin. The observed differences in this study between flocks managed on pasture compared with open range flocks and in flocks with fewer than 500 ewes may be accounted for by greater environmental exposure to *T. gondii* through the presence of feral and domestic felids and rodents on and near pastures and on smaller operations that are less intensively managed than feedlots. Toxoplasmosis is the cause of economic losses to sheep producers across the U.S. and also a threat to human health. Increased producer education is needed to reduce the prevalence of *T. gondii* in sheep operations nationwide.

071

Prevalence of elevated temperatures among horses presented for importation to the United States.

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The objective of this study was to determine the prevalence of and risk factors for elevated body temperature ($T>101.5^0F$) among horses imported into the United States while in quarantine at the three APHIS-approved animal import centers.
Of the 2,062 horses that arrived at New York Animal Import Center (NYAIC) and the 1,600 horses that arrived at the Miami Animal Import Center (MAIC) in 2008, 236 (11.4 percent) and 106 (6.6 percent) had at least 1 elevated temperature, respectively. Of the 1,058 horses that arrived at the Los Angeles Animal Import Center (LA-AIC) in 2009, 127 (12.0 percent) had at least 1 elevated temperature. The prevalence of elevated temperature varied significantly ($p<0.0001$) by import center:
Several models were created to evaluate the potential risk factors for elevated temperatures across centers. Young age (4 years or less) was a risk factor for elevated temperature across all three centers. However the effect of breed categories varied by center. The higher prevalence of horses with an elevated temperature at LA-AIC than at MAIC was explained in part by the fact that there was a higher percentage of young horses (4 years of age or less) at LA-AIC and the prevalence of elevated temperature among young horses entering LA-AIC was very high (60.9%). The higher prevalence of elevated temperatures in horses at NYAIC than at MAIC was explained in large part by the fact that the prevalence of elevated temperatures among Warmbloods entering NYAIC was much higher than among Warmbloods entering MAIC (12.4% vs. 3.6%). The effect of origin country could not be evaluated since the origin of horses varied by center.
The prevalence of elevated temperature varied by import center, as well as by age and breed categories. Determination of the specific causes of elevated temperatures, particularly the distinction between a stress response versus fever due to infection among this population of horses would require collection of additional data.

FOOD AND ENVIRONMENTAL SAFETY

072

Piloting the future: results from a pilot study for changes in the animal sampling program for the national antibiotic resistance monitoring system (narms).

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Purpose: A well recognized monitoring system for antimicrobial resistance in the U. S. is the National Antimicrobial Resistance Monitoring System (NARMS). It was established in 1996 among the Food and Drug Administration (FDA), USDA, and Centers for Disease Control and Prevention (CDC). FDA coordinates the program, collects retail samples, and CDC collects human samples from FoodNet sites. USDA's ARS oversees the animal sampling program for NARMS with collaboration on sample collection from APHIS and FSIS. The goals for the NARMS program are to: monitor trends in antimicrobial resistance from humans, retail meats, and animals, disseminate timely information, conduct research, and spread of antimicrobial resistance, and assist the FDA in making decisions related to the approval of safe and effective antimicrobial drugs for animals.
Recent scientific reviews and reports (including the FDA Science report) have indicated the need for changes in the animal sampling program for the National Antimicrobial Resistance Monitoring System (NARMS). Currently, ARS is conducting a 4 month pilot study to coordinate university and ARS scientists (with industry) to collect animal samples in representative geographical locations and to provide pilot data for FDA on the most representative and cost-effective sampling program. This pilot will be completed in December 2011. This presentation will discuss the challenges of developing an on-farm and slaughter sampling program for antimicrobial resistance in animals. Preliminary data will include sampling approaches, microbiologic methods, and sensitivities for feedlot and dairy cattle, broilers, and turkeys. Swine were not included because they were being sampled in a different pilot. Results will include the challenges, costs and benefits of this pilot and the potential of this program for long term monitoring of antimicrobial resistance in food animals.

073

Prevalence and antimicrobial resistance of potential food safety pathogens on united states beef cow-calf operations

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Purpose: The objective of this study was to determine the prevalence of potential food safety pathogens and their antimicrobial resistance patterns in feces collected on beef cow-calf operations in the United States.
Methods: Fecal samples (n=5,793) were collected from beef cows on 173 operations in 24 states as part of an on-going series of studies conducted by the USDA's National Animal Health Monitoring System (NAHMS). Fecal samples collected during two periods Sample collection occurred over two sampling periods, from January 14 through April 15 and from July 7 through August 31, 2008 were shipped chilled overnight to the USDA:ARS Bacterial Epidemiology and Antimicrobial Resistance research laboratory for culture for *Salmonella*, and *Campylobacter*. In addition samples were cultured for *Enterococcus*, non-type specific *E. coli* and *Clostridium difficile*. All isolates were evaluated for antimicrobial resistance using a semi-automated broth micro-dilution method (*Salmonella, Campylobacter, Enterococcus,* and *E. coli*) or the E-test (*Clostridium difficile*).
Results: Overall, the animal and herd-level prevalence of *Salmonella* was low, 0.5% and 9.2% respectively. The sample and operation-level prevalence of *Campylobacter* was 8.9% and 44.5% respectively with most of the isolates being *C. jejuni*. For *Enterococcus*, *E. faecalis* and/or *E. faecium* were found on 43.9% of operations. Eight different species of *Enterococcus* were identified overall, but 11.5% and 3.2% of samples were positive for *E. faecium* and *E. faecalis*, respectively. *Clostridium difficile* was isolated from 6.3% of samples and on 43.9% of operations. The prevalence of antimicrobial drug resistance varied by organism. All *Salmonella* isolates were pansusceptible to the panel of antimicrobial drugs tested. Most (56.2%) of the *Campylobacter* isolates were susceptible to all antimicrobials tested. For *Enterococcus* isolates resistance was most common to Lincomycin, Flavomycin, and/or Ciprofloxacin.
Conclusions: Prevalence of potential food safety pathogens and their characteristics will be important considerations in assessing the feasibility of potential pre-harvest food safety strategies.

074

The use of multi-level model residuals for food animal disease surveillance.

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Purpose: The residuals from multi-level models used for abattoir condemnation data may contain a wealth of information for food animal syndromic surveillance, yet residuals are underutilized for surveillance purposes. The objective of this study was to examine the results of spatial and space-time scan tests using the residuals from a multi-level Poisson model, thus removing the effect of known confounders from abattoir lung condemnation data. Multi-level model residuals applied to spatial and space-time scan statistics allow the identification of abattoirs performing outside of statistical norms, signaling a potential problem.
Methods: A multi-level Poisson model was constructed using the monthly total slaughtered cattle and the monthly total pneumonic lung condemnations from OMAFRA's Food Safety Decision Support System (FSDSS) from 2001 - 2007. Potential confounding seasonal, secular, disease and non-disease factors were accounted for in the model to remove "noise" in the data. The 1st and 2nd level standardized residuals were used from the multi-level model in spatial and space-time scan tests using a normal model to identify abattoirs in space or space-time which may be condemning unusually high or low rates of pneumonic lungs.
Results: The results of the spatial scan of the 2nd level residuals identified one low rate cluster in southern Ontario. In comparison, the space-time scan of the 1st level residuals identified 3 high rate clusters in western and southern Ontario during 2001-2004, and 1 low rate cluster in central Ontario from 2001-2003.
Conclusions: After controlling for various confounding factors, we are still identifying higher pneumonic lung condemnation rates than would be expected. In future applications, this approach may assist in identifying potential outbreaks or in auditing the performance of abattoirs.

075

Vaccination to control *Escherichia coli* O157 in integrated cattle production systems.

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Purpose*: E. coli* O157 (*EcO157*) causes significant illness in the U.S. and Argentina. Immunization of cattle against siderophore receptor and porin (SRP®) proteins has been proposed as a control strategy. Our objective was to evaluate whole-herd vaccination with a SRP-based vaccine using 2- and 1-dose regimens. Methods: In *Study A*, 10 feedlots were enrolled as vaccinate yards within which all animals were administered at least 1 dose of the SRP *EcO157* vaccine. Animals harvested from May through August were administered 2 doses. Feces were collected from 5 vaccinate and 5 control feedlots. Two-dose

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vaccinates were harvested in a single abattoir and time-matched non-vaccinates served as controls. Hide swabs, sera and trim testing was performed to evaluate an association with vaccination.In *Study B*, all cattle in 2 feedlots were administered 1 dose and 2 matched feedlots served as controls. Fecal samples were collected from each feedlot twice. In an abattoir, hide swabs and trim testing was performed to test an association with vaccination. Results: In *Study A*, prevalence of *EcO157* in feces from vaccinates was lower ($P \leq 0.01$) than controls and was 12.8 and 20.4% ($P<0.01$). Vaccination did not reduce prevalence on hides but was associated with fewer units of trim associated with *EcO157* ($P=0.04$). Titers to vaccine antigens were greater among vaccinates (0.62) than controls (0.08; $P<0.01$). In *Study B*, prevalence during the initial visit was lower ($P=0.08$) among vaccinates than controls but did not differ during the second visit ($P=0.61$). Prevalence on hides of vaccinates and controls was 12.2 and 25.8% ($P<0.01$). No difference among routine testing of trim was evident (i.e., only 1 total positive). Conclusions: These data indicate that while the SRP-based *EcO157* vaccine did not eliminate *EcO157*, it was associated with reduced prevalence in various metrics including a microbiological association with presumptive positives in beef trim. Because of observed herd immunity, we adopted whole-herd regimens which may have failed to adequately control confounding variables; yet because *EcO157* is a commensal organism, the impact of confounding is uncertain and potentially of limited influence.

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Prevalence of Shiga toxin-producing Escherichia coli (STEC) genes by multiplex PCR in cattle and their environment, Michigan 2011.
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Purpose: STEC is a leading cause of human food-borne disease outbreaks and cattle are a major reservoir. The objectives of this study were to determine the prevalence of STEC in dairy and beef herds in environmental and individual animal samples and to compare alternative ways of sampling the environment.
Methods: A total of 75 environmental samples were collected from 3 dairy herds and 3 beef herds from adult cow pen floors, manure storage areas, drinking water and water sediments, and boot covers worn while walking through cattle environments. A total of 608 animals were sampled by recto-anal junction (RAJ) swabs. A subset of 71 animals was sampled longitudinally (3 times every 2 weeks) by swabbing the RAJ and collecting rumen fluid. Specimens were incubated in Gram negative broth overnight, and crude DNA was extracted for multiplex PCR targeting Shiga toxin (*stx1, stx2)* and intimin (*eae*) genes.
Results: At least one *stx* gene was detected in 43.3% (13/30) of the pen floor samples collected, while the manure storage samples and boot covers were positive in three of the six herds. In all herds, water sediment samples (n=16; 43.8 %) were more likely to be positive than drinking water samples (n=16; 18.8%). Of the individual animals sampled; 5.9% were positive for *stx1*, 48.5% for *stx2*, and 7.0% were positive for *stx1,2*. In total, 61.5% (374/608) of animals were positive for *stx* in the RAJ, while 45.9% (67/146) of the rumen fluid samples were positive. PCR prevalence was higher in dairy cattle (n=297/421; 70.5%) than in beef cattle (78/187; 41.7%).
Conclusions: In summary, there was a high PCR prevalence of the *stx* genes in both environmental samples and RAJ samples of individual animals. Future work is required, however, to recover STEC from PCR positive samples to determine whether the PCR prevalence accurately predicts STEC prevalence in a given herd. It is important to determine more effective ways of sampling and to understand temporal changes in shedding levels. The results of this research could guide new strategies aimed at reducing STEC contamination of beef products.

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Multiple-locus variable-nucleotide tandem repeat analysis of *Escherichia coli* O157:H7 evaluating isolate distribution on a closed feedlot facility, Wooster, OH.
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Purpose: Despite increased research, foodborne transmission of *Escherichia coli* O157:H7 from cattle is still a vital concern. An important aspect to consider for controlling *E. coli* O157 on the farm is the epizootiology of different isolates over time. As a follow-up to a previous study to assess prevalence of *E. coli* O157:H7 and supershedder status in feedlot cattle fed different energy sources (high moisture corn vs dry whole-shelled corn) and feed additives, we characterized the positive *E. coli* O157:H7 isolates by multiple-locus variable-nucleotide tandem repeat analysis (MLVA) to assess the community composition within a growing season.
Methods: Isolates confirmed as *E. coli* O157 by the presence of O157 antigen were subsequently subtyped by MLVA using the standardized protocol available from PulseNet.
Results: We identified at least three distinct MLVA subtypes (A, B, C) present on the feedlot during the study, accounting for 25%, 15% and 60% of total isolates recovered throughout the feeding period respectively. Subtypes A and B were isolated on the first 2 sampling dates but their prevalence waned as the study progressed. Subtype C, first isolated on sampling date 3, became the predominate subtype on the feedlot. Supershedder status was associated with all three subtypes supporting the idea that supershedding is a function of the infection process and not the isolate subtype.
Conclusions:Understanding the epizootiology and the on-farm transmission dynamics of *E. coli* O157:H7 may aid in the development of pre-harvest control strategies.

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Intimin type characterization of non-O157 Shiga toxin-producing *Escherichia coli* isolates.
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Purpose: Non-O157 Shiga toxin-producing *E. coli* (STEC) are enzootic in cattle and constitute an emerging zoonotic threat. A current research goal of the USDA-NIFA is the development of pre-harvest interventions for STEC O26, O45, O103, O111, O121, O145, and O157:H7. Preliminary studies were conducted to characterize the STEC isolates from each of these seven serogroups for intimin type since this may influence the site and extent of intestinal colonization, and thereby constitute a suitable target for vaccines or other intervention strategies.
Methods: The intimin gene (*eae*) type of 25 human, bovine, caprine and environmental STEC isolates divided among serogroups O26, O45, O103, O111, O121, or O145 were characterized by polymerase chain reaction (PCR) and nucleotide sequencing. Isolates were first screened for the presence of *eae* using primers that targeted the conserved 5' region, and subsequently tested for *eae* type (α-ξ) using primers that targeted the variable 3' region. Amplicons of the expected size for a given *eae* type were then sequenced for confirmation.
Results: Twenty-two of 25 isolates (88%) were PCR-positive for *eae*; of the 3 that tested negative, 2 were O121 and 1 was O111. Based on type-specific PCR and confirmation by nucleotide sequencing, 3 of 3 O26 isolates contained *eae*-β1; 4 of 4 O45 isolates contained *eae*-ε; 5 and 1 of 6 O103 isolates contained *eae*-ε and *eae*-β1, respectively; 1 of 1 O111 isolates contained *eae*-β1; and 1 and 2 of 3 O145 isolates contained *eae*-β1and *eae*-γ1, respectively. The *eae* types of 4 positive isolates (1 O103 and 3 O111) have not yet been determined.
Conclusions: The *eae* gene was common among the non-O157 STEC isolates tested. The prevalence of *eae* among isolates tested herein was similar to that

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of another study in which 84% of non-O157 isolates possessed *eae*. Purification and sequencing of selected PCR products was necessary to determine the intimin type.

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Multistate Markov chain model to describe and compare fecal shedding dynamics of three *Escherichia coli* O157:H7 strains in cattle.
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Our objective was to compare fecal shedding dynamics for three *Escherichia coli* O157:H7 strains of different genotype (FRIK47, FRIK1641, and FRIK2533) using data from experimentally inoculated steers. FRIK47, also known as the human EDL933W isolate, has shiga-toxin and enterohemolysin genes; FRIK1641 also has enterohemolysin genes but shiga-toxin genes may or may not be present, while FRIK2533 has shiga-toxin genes but lacks the enterohemolysin genes. A total of 24 steers were randomly inoculated with the three strains: twelve with FRIK47 and 6 steers each with FRIK1641 and FRIK2533. Fecal samples were collected the day after inoculation and every two days thereafter until 30 days post inoculation to monitor shedding of *E. coli* O157:H7 in feces. To compare the dynamics of fecal shedding for the three strains, a multistate Markov chain model was developed. The model included 3 transient (representing latency, shedding, and non-shedding) states and one absorbing state representing recovery. Shedding of the three strains was different. There was no shedding among steers inoculated with FRIK2533 except for two steers that shed only on the second day following inoculation. The other two strains were shed repeatedly by most inoculated animals. The risk of progression from latency and shedding to recovery for FRIK47 was lower compared to FRIK2533 (hazard ratio (HR=0.05, 99% CI= 0.01, 0.28) and FRIK1641 (HR=0.47, 80% CI= 0.22, 0.99), at a significance level of 0.01 and 0.2, respectively. Slower progression to recovery suggests a longer overall duration of host infection with FRIK47 than with the other two strains. Indeed, the average total time spent in the shedding state during infection was considerably higher for FRIK47 (15 days) compared to FRIK2533 (1 day) and FRIK1641 (4 days). Considering the crucial role that pathogen shedding has on infection transmission, these findings suggest that *E. coli* O157:H7 genotype may affect its transmissibility in the host population. Furthermore, the existing genetic diversity of *E. coli* O157:H7 in cattle herds may partially explain the variability in the observed herd level prevalence in *E. coli* O157:H7 fecal shedding.

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Characterization of *Escherichia coli* carrying bla_{CTX-M} isolated from fecal flora of dairy cattle.
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First reported in fecal *E. coli* of US livestock in 2010, CTX-M extended spectrum β-lactamase genes encode for the production of enzymes capable of inhibiting the antimicrobial effects of important cephalosporin drugs. This plasmid-borne gene conveys resistance to the penicillins as well as 1st, 3rd, and 4th generation cephalosporins, but not to cephamycins or β-lactamase inhibitors. We characterized a collection of 70 PCR-confirmed fecal *E. coli* isolates carrying bla_{CTX-M} recovered from 5 Ohio dairy herds at the level of the bacterium, plasmid, and gene. Isolates were evaluated by PFGE and minimum inhibitory concentration (MIC) to compare and contrast bacterial relatedness and antimicrobial resistance phenotype. Plasmids were conjugated to a K12 MG1655 recipient, extracted and analyzed by restriction fragment analysis using *Acc*I. Plasmid replicon types were determined using multiplex PCR. Gene amplicons were bi-directionally sequenced and analyzed using BLAST.
Multiple CTX-M genes were recovered including CTX-M-1, -15, and -14, with specific genes clustering within herds. PFGE of bacterial backbones found similar within herd clustering of isolates with the exception of one herd which contained at least 6 unique bacterial fingerprints. Isolates were resistant to ampicillin, cefazolin, cephalothin, cefotaxime, cefpodoxime, ceftriaxone, ceftiofur, and cefepime, and had ceftazidime MICs which ranged from 2 to 8 ug/ml. These isolates were susceptible to cefoxitin and to β-lactam drug combinations that included clavulanic acid or tazobactam. An exception was isolates from one herd which were found to harbor the AmpC β-lactamase gene bla_{CMY-2} in addition to the CTX-M.

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Validation of culture methods for non-O157 Shiga toxin-producing *Escherichia coli*.
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Purpose: Non-O157 Shiga toxin-producing *E. coli* (STEC) are enzootic in cattle and constitute an emerging zoonotic threat. A current research goal of the USDA-NIFA is the development of diagnostic tests for STEC O26, O45, O103, O111, O121, O145, and O157:H7. Preliminary studies were conducted to test the validity of a published culture protocol (Possé et al. 2008. FEMS Microbiol. Lett. 282:124-131) for detection of STEC O26, O103, O111, O145, and O157:H7, and to determine whether it can be used for detection of O45 and O121. In addition, we tested the validity of Rainbow® O157 Agar NT for detection of all seven serogroups, since it has been recommended for that purpose by the USDA-FSIS (MLG 5B.00).
Methods: We tested these media on 56 different strains, eight each of serogroups O26, O45, O103, O111, O121, O145 and O157:H7, in pure culture.
Results: Compared to the nutrient base without selection [trypticase soy broth (TSB)], strains grown in selective enrichment medium (TSB-NVRBT) had significantly reduced growth; this was true for all non-O157 STEC (O26, O45, O103, O111, O121, O145) and O157:H7 STEC tested. Growth of STEC O26, O103 and O121 strains was especially reduced. The results of non-O157 STEC culture on Possé differential medium were not entirely as expected; however, all seven serogroups (O26, O45, O103, O111, O121, O145, and O157:H7) were represented by red-purple, blue-purple, blue-green, and green colonies. The confirmation medium described by Possé et al. did not perform as expected, and was not reliable. Rainbow® O157 Agar NT, although currently recommended by the USDA-FSIS-MLG 5B.00, was unreliable for non-O157 STEC detection.
Conclusions: The results suggest that the differential culture medium described by Possé et al. (FEMS Microbiol. Lett. 2008;282:124-131) can be used to concurrently screen for all seven serogroups (O26, O45, O103, O111, O121, O145, and O157:H7). More research on the development and validation of detection methods for non-O157 STEC is needed.

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Simulation model of vaccinating cattle against STEC O157 for pre-harvest food safety.
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Purpose: Observed data indicate pens of cattle with high *Escherichia coli* O157:H7 (STEC O157) prevalence at harvest present a greater risk to food safety than pens of lower prevalence. Vaccination of live cattle against STEC O157 has been proposed as an approach to reduce STEC O157 prevalence in live cattle. A stochastic model simulates reality under conditions of uncertainty. Our objective was to create a stochastic simulation model (@Risk, V 5.7.1), to compare STEC O157 prevalence distributions for summer- and winter-fed cattle to summer-fed cattle immunized with a 3-dose regimen of Type III

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secreted protein (TTSP) vaccine.
Methods: Model inputs were an estimate of vaccine efficacy, observed frequency distributions for number of animals within a pen, and pen-level fecal shedding prevalence for summer and winter. Uncertainty about vaccine efficacy was simulated using a log binomial distribution from a previous performed meta-analysis of four randomized controlled 3-dose regimen TTSP vaccine trials. The outcome was post-vaccination STEC O157 fecal pen prevalence. The simulation was performed 5,000 times.
Results: Summer fecal prevalence ranged from 0% to 80% and averaged 30% (std dev ±0.24). Forty-one percent of summer-fed pens had STEC O157 prevalence greater than 30%. Winter fecal prevalence ranged from 0% to 60% and averaged 10% (std dev ±0.13). Seven percent of winter-fed pens had STEC O157 prevalence greater than 30%. Fecal prevalence for vaccinated pens of summer-fed cattle ranged from 0% to 54% and averaged 16% (std dev ±0.13). Sixteen percent of vaccinated pens had STEC O157 prevalence greater than 30%. The simulation outcome was most sensitive to the shedding prevalence of control pens of cattle.
Conclusions: In this simulation vaccination mitigated the risk STEC O157 fecal shedding to levels comparable to winter, with the major effect being reduced variability in shedding prevalence. Food safety decision-makers may find this information useful for evaluating the value of pre-harvest interventions. The simulation model should prove useful for evaluating other STEC O157 pre-harvest interventions.

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Virulence profiling of Shiga toxin-producing *Escherichia coli* O111:NM isolates from cattle.
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Molecular characterization of important non-O157 Shiga toxin-producing *Escherichia coli* (STEC) STEC has lagged considerably behind that of O157:H7 strains. The purpose of this study was to characterize STEC O111:NM isolates of cattle origin. Fifty-eight STEC O111:NM isolates were characterized by PCR. The isolates were screened for the presence of 25 genes that encode virulence factors and markers. These included Shiga toxins (*stx1* and *stx2*), intimin (*eae*), plasmid encoded genes *(ehxA, katP* and *espP*), pathogenicity islands (OI) markers for OI-43/48 (*iha, ureC, terC*), and OI-122 (*Z4321, Z4326, Z4332, Z4333*). Virulence profiling revealed that 100% (58/58) possessed *stx1* and *eae*, and 18.9% (11/58) carried *stx2* as well. The distribution of plasmid-encoded putative virulence genes was as follows: *ehxA*, 75% (44/58); *katP* 41% (24/58); *espP*, 51 (30/58). The frequencies of the four OI-122 marker genes in STEC O111:NM were 57% (33/58) for *Z4321* and 100% for *Z4326*, *Z4332*, and *Z4333*. The full complement of OI-122 markers was present in 57% of the STEC O111:NM strains tested. The distribution of OI-43/48 markers was 100% (58/58) for *terC*, *ureC*, and *iha*. Non-LEE encoded effector genes were distributed as follows: *nleA*, 36% (21/58); *nleB*, 100% (58/58), *nleC*, 0% (0/58); *nleE*, 100% (58/58) *nleF*, 7% (4/58); *nleG2-1*, 71% (43/58); *nleG2-3*, 100% (58/58); *nleG5-2*, 100% (58/58); *nleG6-2*, 62% (36/58); *nleG9*, 95% (55/58); *nleH1-1*, 100% (58/58); *and nleH1-2*, 91% (53/58). Major conclusions are that the majority of STEC O111:NM from cattle that were characterized in this study possessed the *stx1, eae* and *ehxA genes*. However, most of STEC O111:NM lack *stx2*, a major virulence gene that has been associated with severe disease in humans. With regard to OI-122 and OI-43/48 and *nle* marker genes, more than 50% of STEC O111:NM isolates possessed these genes. STEC that possess the full spectrum of *nle* marker genes and complete OIs in combination with *stx2* are most likely to be incriminated in outbreaks of human disease. To the best of our knowledge, this is the first study that has characterized a large number of STEC O111:NM beyond the more traditionally recognized virulence factors and markers (*stx, eae* and *ehxA*).

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Meta-analysis of a three-dose regimen of a type III secreted protein vaccine for efficacy at reducing STEC O157 in feces of feedlot cattle.
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Purpose: Pre-harvest control of *Escherichia coli* O157:H7 (STEC O157) may prevent human illness through decreased carriage by live cattle and reduced transmission to carcasses, or the environment. Immunization of cattle with a type III secreted protein (TTSP) vaccine inhibits colonization of cattle with STEC O157. Our objectives were to perform a meta-analysis to estimate efficacy of a three-dose regimen of TTSP vaccine at reducing the presence of STEC O157 in the feces of feedlot cattle.
Methods: Pen-level data (n=182 pens, 1,462 cattle) from four natural exposure randomized controlled vaccine trials conducted from 2002-2008 at the University of Nebraska-Lincoln Research Feedlot (Mead, NE) were analyzed. Factors explaining the probability for a positive fecal sample were tested in a generalized estimating equations (GEE) logistic regression model. An autoregressive correlation structure was defined to account for clustering of repeated test-periods within block. Clustering or potential confounding by study was accounted for by treating study as a fixed effect. Relative risk was estimated from a corresponding log-binomial GEE model.
Results: Over all studies, STEC O157 was detected from 661 of 5,451 post-vaccination samples. The probability to detect STEC O157 post-vaccination was 8.4% and 15.8% in vaccinated and unvaccinated cattle, respectively. Interactions between vaccination and 1) study; 2) prevalence of control pens within each time-place cluster; and 3) days from vaccination were not significant or fit poorly with observed data. Adjusting for study, cattle in pens receiving three doses of vaccine were less likely to shed STEC O157 (OR=0.46 p<0.0001). Model adjusted vaccine efficacy was 48% (95% CI, 0.37 - 0.57).
Conclusions: We concluded that a three-dose regimen TTSP vaccine is efficacious at reducing the probability to detect STEC O157 in the feces of cattle.

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Antimicrobial resistance in *Escherichia coli* recovered from feedlot cattle.
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The objectives of this study were to estimate prevalence of antimicrobial resistance in feedlot cattle produced under typical commercial conditions, and to investigate associations between antimicrobial use (AMU) and antimicrobial resistance (AMR). Cattle housed in 310 pens at four large feedlots in Canada were randomly selected for enrollment. Pooled fecal samples were collected from pen floors on 1-4 occasions during the feeding period. Up to 5 *E. coli* isolates were selected from each culture and 8,644 isolates were analyzed for susceptibility to 19 antimicrobials by two independent laboratories using broth microdilution and disk diffusion. Records of antimicrobial use were obtained from computerized treatment records. Prevalence of AMR and 95% confidence intervals were estimated for each time period using alternating logistic regression, categorizing data by days-on-feed (DOF) at sampling. Associations between AMU and AMR were assessed at the pen level using multivariable logistic regression and GEE to control for clustering. Prevalence of resistance to 3/19 drugs evaluated was >10% during at least 1 interval during the feeding period. Resistance in isolates obtained at arrival had a resistance prevalence to streptomycin of 19.7%, to sulfisoxazole of 19.3%, and to tetracycline of 55.2%. In samples collected 1-75 days on feed (DOF), resistance prevalence had increased to 25.4%, 23.9% and 71.8%, respectively. Samples obtained 75-120 DOF showed a streptomycin resistance prevalence

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of 24.4%, a sulfisoxazole resistance prevalence of 26.4% and a tetracycline resistance prevalence of 76.0%. For samples obtained >120 DOF, resistance prevalence had decreased to 17.6% and 17.2% for streptomycin and sulfisoxazole, respectively, while resistance to tetracycline increased to 81.1%. There was limited evidence of associations between pen-level metrics of antimicrobial exposures and the occurrence of AMR. Using pooled samples is an efficient method for estimating resistance prevalence in feedlot cattle, but limits the ability to investigate AMU as a driver of AMR in individual cattle. This may not be a major limitation if AMU in the feedlot is not an important determinant of AMR.

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Effects of a vaccine and a direct-fed microbial on fecal shedding of *E. coli* O157:H7 in pens of commercial feedlot cattle fed a diet supplemented with distiller's grains.
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Our objective was to determine the efficacy of a siderophore receptor and porin (SRP®) proteins-based vaccine (VAC) and a direct-fed microbial (DFM; Bovamine®) for controlling fecal shedding of *E. coli* O157:H7 in pens of feedlot cattle fed a corn-based diet with 25% distiller's grains. Cattle projected for harvest during the summer were randomly allocated into 40 pens grouped by arrival dates into 10 complete blocks; pens within block were randomly allocated to a control, VAC, DFM, or VAC+DFM treatment. The DFM was fed (10^6 CFU/animal/day) throughout the study periods (approximately 80 days) and vaccine was administered on days 0 and 21. Fresh fecal samples (30/pen) were collected weekly from pen floors for four consecutive weeks prior to projected harvest. Laboratory personnel blinded to treatments used both immunomagnetic separation and semi-quantitative direct plating (for high shedders; $\geq 10^4$ CFU/gram) procedures to isolate *E. coli* O157:H7. Data were analyzed using generalized linear mixed models that accounted for clustering. Overall, sample-level prevalence was 31.7% and high shedder prevalence was 3.5%. For either measure of *E. coli* O157:H7 shedding, no significant effects were observed for treatment and sampling time interaction, VAC and DFM interaction, or DFM. However, vaccinated pens had a significantly ($P < 0.05$) lower overall prevalence (model-adjusted mean (± SE) = 17.4% (3.9)) and lower prevalence of high shedders (1.0% (0.3)) than non-vaccinated pens (37.0% (6.3) and 4.2% (0.8), respectively). We conclude that the SRP vaccine can reduce fecal shedding of *E. coli* O157:H7 in potentially high risk populations of feedlot cattle.

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Fecal shedding of *Escherichia coli* O26 in feedlot cattle from a field trial evaluating an *Escherichia coli* O157:H7 vaccine and a direct-fed microbial.
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Escherichia coli serogroup O26 is second only to O157 as a cause of Shiga toxin-producing *E. coli* infections. Our objective was to determine the prevalence and virulence genes of *E. coli* O26 in feces of commercial feedlot cattle that were enrolled in a field trial evaluating an *E. coli* O157:H7 siderophore receptor and porin (SRP®) proteins-based vaccine (VAC) and direct-fed microbial (DFM; Bovamine®). Cattle were randomly allocated to 40 pens within 10 complete blocks; pens were randomly assigned to control, VAC, DFM, or VAC+DFM treatments. Vaccine was administered on days 0 and 21, and DFM was fed (10^6 CFU/animal/day) throughout the trial. Pen floor fecal samples (30/pen) were collected weekly for 4 consecutive weeks prior to projected harvest. Samples were enriched in *E. coli* broth, subjected to immunomagnetic separation, and plated on MacConkey agar. Ten colonies were randomly picked, pooled and tested by multiplex PCR for O26 and *stx*1, *stx*2, *eae*, and *hly*A genes. Overall sample-level prevalence of O26 was 22.7 %; however, only 22.5% of the O26-positive samples were positive for *stx* genes. Preliminary analysis demonstrated no significant effects of treatment, or treatment by sampling week interaction, on O26 prevalence. However, a significant ($P < 0.01$) sampling week effect was observed with higher O26 prevalence in the two weeks prior to harvest compared to the previous two weeks. Preliminary results suggest the treatments had no significant effect on O26 shedding; however, these results are based on pooled colonies and ongoing analysis of individual isolates should provide further detail.

088
Development of a semi-quantitative ranking scheme to estimate the concentration of *Escherichia coli* O157:H7 in bovine feces.
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Purpose: Methods for quantification of *E. coli* O157:H7 in bovine feces are needed, but vary with intent, precision, cost, labor and time. Our objectives were to compare a semi-quantitative ranking scheme to traditional direct plating and most probable number (MPN) methods to quantify E. coli O157 in feces, and to characterize time-dependent changes in concentration of *E. coli* O157 in samples stored at 4°C.
Methods: Feces collected in 2010 and 2011 from commercial feedlots were cultured for *E. coli* O157:H7. Positive plates from immunomagnetic separation (IMS) were assigned a rank (1-5) according to number of colonies (1=1-10, 2=11-20, 3=21-30, 4= 30+, 5=Lawn). From these positive samples (n=460) 1g or 11g samples were serially diluted and direct plated to CHROMagar™ and incubated for 18 hours at 37°C for quantification and another set of positive samples (n=111) were run in a 3x5 MPN configuration.
Results: Concentration was associated with a quadratic rank term (P<0.01). On average, ranks 4 and 5 were associated with quantifiable direct plating whereas ranks 1 through 3 were near or below the detection limit of direct plating. Mean values for ranks 1-5 were; 0.47, 0.44, 0.66, 1.07 and 3.12, respectively. Mean MPN values for ranks 1-5 were; 0.03, -0.03, 1.15, 1.22, and 3.05, respectively. Collapsing of ranks (1,2=1, 3,4=2, 5=3) resulted in direct plate mean values of 0.46, 0.99 and 3.12, respectively while mean MPN values were 0.01, 1.21, and 3.05 for ranks 1-3 respectively. A subset (n=69) were stored at 4°C and subjected to re-assessment over a 10 week period, 11 samples of which were ranked 4 or 5 and direct-plated during each re-assessment. Positive detection by IMS had decreased 49% by week 2 and 81% by week 10. The ability to detect via direct plate decreased by 50% at week 2 while overall count averages decreased by 99.99% by week 10. Similarly, IMS rank averages decreased over the sampling duration.
Conclusions: These data indicate the semi-quantitative method may be used as a convenient and repeatable proxy for quantification while decreasing costs and labor and indicate fecal samples should be analyzed sooner than 2 weeks at 4°C.

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Modeling the effect of bacterial transfer rates and interventions on the prevalence and concentration of *Escherichia coli* O157 on beef carcasses.
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Purpose: *Escherichia coli* O157 is a foodborne pathogen with important economic implications to the beef industry. Although considerable progress has been made in reducing the presence of *E. coli* O157 in meat products, outbreaks still occur. The importance of specific events along the production continuum influence commercial practices, and understanding the relative importance of each event is valuable for future decision making. Our objective

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was to model the effects of post-harvest interventions as well as the influence of bacterial transfer between feces, hide, and carcasses on the prevalence and concentration of *E. coli* O157 in U.S. beef cattle carcasses at harvest.
Methods: We constructed a risk assessment model using @Risk in Microsoft Excel. Parameter distributions were developed from the existing scientific literature and incorporated into a Monte-Carlo framework. The prevalence and concentration of *E. coli* O157 on beef cattle carcasses were conditional on fecal prevalence estimates, high shedder prevalence estimates, transportation and lairage effects, hide and carcass intervention(s), and the relationship between high shedders and hide contamination and hide to carcass bacterial transfer. We assessed the prevalence and concentration of *E. coli* O157 on carcasses based on a standard truckload of 40 head of cattle. Multiple scenarios were evaluated and each scenario was run for 50,000 iterations.
Results: Our results indicate that the percentage of bacteria transferred between hides and carcasses is influential to carcass prevalence and concentration; however, there is little data to inform the distribution of this transfer. As expected, the sensitivity analysis showed successful carcass intervention has the highest impact on final carcass prevalence and concentration. The results of our model also support higher prevalence and concentration of carcass contamination when multiple factors fail within the system.
Conclusions: This "perfect storm" results in the rare occurrence of elevated carcass prevalence and *E. coli* O157 contamination levels on individual or multiple carcasses within lots and highlights the need for multiple intervention points.

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Assessing risks of microbial contamination of produce from irrigation water.
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Purpose:The increased reports of foodborne illness related to fresh produce in recent years has spurred interest in irrigation water as a potential route of vegetable contamination worldwide. The aim of the study was to provide data to establish science-based guidelines for stakeholders to mitigate the risk of irrigation-borne microbial contamination of produce.
Methods:Relevant studies involved: 1) Determination of the variability of microbial indicators (coliforms, E. coli) over an irrigation period to ascertain the sampling frequency required to accurately assess the quality of water typically present at each of six separate surface water sources used for irrigation; and 2) Determination of the association between microbial indicators in irrigation water and on edible plants. For the first study, 196 samples were collected from four reservoirs and two irrigation ditches and tested. Bootstrap analysis was applied to determine the sensitivity of the measured parameters to variation in sampling frequency. In the second study, data analyzed included 150 water: vegetables pairs collected in Ohio, US, and 14 samples of surface irrigation water and tomatoes in the field (n=34) in Kaduna State, Nigeria.
Results:From the first study, water in irrigation ditches was of lower quality than that in reservoirs and increased significantly following heavy rainfall events. The 95% confidence intervals surrounding the estimate based on a single sample were wide, surpassing the upper limit for acceptable use for irrigation purposes recommended by several organizations. From the second study, there were no significant associations between fecal indicators in water and on vegetables, in either the US or Nigerian samples sets.
Conclusions:In conclusion, a single measurement of irrigation water quality imprecisely reflected the quality of water over the course of the irrigation period. Moreover, this number was not correlated with the magnitude of the same organisms present on edible plants. Guidelines that recommend a single irrigation water quality test as measure microbial food contamination risks lack strong scientific justification.

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Inactivation kinetic of feline calicivirus (norovirus surrogate) on lettuce by electron beam irradiation.
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Norovirus is a major viral source for acute gastroenteritis in human. More than 20 million cases of norovirus infection have been reported each year in the US. While noroviruses spread frequently through foods or water contaminated by affected humans, conditions in the production environment may also result in contamination. Since produce, such as vegetables and fruits, are commonly consumed without additional processing except a brief water rinse, consumers are at higher risk of norovirus infection if purchased products are contaminated. It is important to evaluate the intervention method to reduce the presence of human pathogens in such products. Irradiation has been approved for lettuce and spinach to control bacterial contaminants and can be a safe decontamination strategy for viral contaminants. The objective of the study was to determine the relationship between electron beam irradiation and the inactivation of norovirus on leafy green vegetables. Feline calicivirus (FCV) was used as surrogate for small round structured viruses including norovirus on lettuce. Cut lettuce (100g) was inoculated with the virus (106 TCID50 in 100ml) by immersion to simulate contamination from irrigation or wash water and exposed to electron beam irradiation at dose of 0 to 5 kGy. Irradiated lettuce samples were then titrated in CRFK cells to determine virus survival. A reverse liner dose response was observed between irradiation dose and virus titer in lettuce. An irradiation dose of 1 kGy appeared to reduce the population of FCV by approximately 100.33 TCID50/g. The dose of irradiation required to reduce FCV titer on lettuce by 90% was approximately 2.95 kGy which was within the FDA approved dose. In conclusion, low dose ionizing radiation has the potential to improve the safety of leafy green vegetables by reducing both bacterial and viral pathogens

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Effects of feeding copper sulfate, tetracycline and tylosin on the prevalence of transferable copper resistance gene, *tcrB*, among fecal enterococci of swine.
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Purpose: Copper sulfate is often used as a growth promoter in swine production. Earlier work has illustrated the presence of the transferable copper resistance (*tcrB*) gene among swine enterococci and its co-location with *erm*(B) and *tet*(M) genes on the same transferable plasmid. The present study was undertaken to determine the effects of single or multiple feed grade antimicrobials on selection and co-selection of *tcrB*-positive enterococci in piglets.
Methods: The study consisted of 240 weaned piglets, housed in groups of 5 animals per pen (n=48). The pens were randomly allocated to six treatments (8 pens per treatment), arranged as an incomplete factorial design, comprising basal diets supplemented with none (control), copper (Cu), chlortetracycline (CTC), tylosin (Tyl), copper and tylosin (CuTyl), or copper and chlortetracycline (CuCTC). The treatment phase was for 4 weeks followed by a washout phase for two weeks. Fecal samples were collected on days 0, 7, 14, 21, 28, and 35. All the enterococcal isolates were tested for the presence of *tcrB* gene by PCR. An equal number of *tcrB*-positive and matched-negative isolates (by pen, date, and treatment) were also tested for both *erm*(B) and *tet*(M) genes.
Results: A total of 372 enterococcal isolates were positive for the *tcrB* gene with an overall prevalence of 14.4% ($P = 0.003$). The prevalence of *tcrB*-positive enterococci in each treatment group was: control (47/432; 10.8%), Cu (52/432; 12.0%), CTC (79/432; 18.3%), Tyl (51/432; 11.8%), CuCTC (75/432; 17.4%), and CuTyl (68/432; 15.7%). The *tcrB*-positive isolates had a mean copper MIC of 17.8 mM, when compared to *tcrB*-negative isolate with

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an MIC of 6.6 mM. All the *tcrB*-positive and matched-negative isolates also carried both *erm*(B) and *tet*(M) genes with phenotypic resistance to erythromycin and tetracycline respectively.
Conclusions: The supplementation of additional antimicrobials had an additive effect beyond what would have been expected as a simple substitution and showed higher prevalence of *tcrB* gene when compared to copper alone. Further studies are being undertaken to study both the phenotypic and genotypic differences among other enterococcal isolates of the present study.

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Export risk assessment for the export of deboned beef from South Africa to Egypt.
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Purpose: Member states of the World Trade Organization (WTO) cannot impose trade restrictions on trade in animal products without valid scientific evidence. In this study we undertake a quantitative risk assessment of introducing the Foot and Mouth Disease (FMD) virus into Egypt by exporting deboned beef from South Africa. Results of this study can be used as a negotiation tool to allow South Africa export deboned beef to Egypt.
Methods: A risk analysis model was developed based on the anticipated uncertainties. Monte Carlo risk analysis using @RISK was used to simulate the dependencies between model uncertainties and to estimate the risk. Data used in the model were obtained from papers in peer reviewed journals, and expert opinions. The triangle distribution was mostly used to describe the uncertainty of the problems.
Results: The expected probability to export one undetected deboned FMD infected carcass to Egypt annually was estimated to be $4.6*10^{-8}$ (90% confidence interval: $6.1*10^{-8}$, $129.42*10^{-8}$) . We estimated that it would take 224 years (90% confidence interval: 53, 1109) before one undetected FMD infected carcass is exported to Egypt. Based on the model, the elements that contributed the greatest uncertainty were: the number of FMD outbreaks per year, and failure of the maturation/deboning process. The failure to detect FMD cases during Surveillances contributed the least uncertainty.
Conclusions: The Sanitary and phyto-sanitary measures that South Africa employs to ensure exported deboned beef is free of the FMD virus are effective. The risk of exposing the national cattle herd of Egypt to the FMD virus through deboned beef imports from South Africa is very low. South Africa needs to put more emphasis on implementing measures that can minimize the frequency of FMD outbreaks in the country. Controls at the abattoir should ensure that carcasses attain the right temperature and pH during maturation. To further minimize the risk of introducing the FMD virus to Egypt through imported beef, we recommend that the maturation process, deboning, removal of blood clots and lymph nodes, be made Critical Control Points (CCPs) in all export HACCP certified abattoirs in South Africa.

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Detection of *Salmonella enteritidis* in poultry environmental samples using a pooled real-time PCR assay.
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Introduction: Real-time (RT) PCR assays have been developed in recent years as a rapid testing methodology for the detection of *Salmonella enteritidis* (SE) in a variety of matrices. Numerous studies have been conducted to examine the efficacy of pooling environmental samples for testing by culture. However, very little research has been done with RT PCR to examine whether the same level of accuracy can be found in pooled samples as opposed to individual samples. Purpose: To determine the equivalency of an SE-specific RT PCR in pooled environmental samples to individual sample testing by RT-PCR and culture. Methods: Three sets of environmental field samples were collected from several poultry facilities previously found to be positive for SE. Set 1 (84 samples) was cultured by the FDA BAM method while Set 2 (108 samples) and Set 3 (100 samples) were combined and cultured by NPIP guidelines. Post- incubation buffered peptone water (Set 1) and tetrathionate (Set 2, Set 3) aliquots were collected and tested by RT PCR individually and in pools of two, three and four. Cut-off (Ct) values were selected and evaluated for improvement in agreement between culture and RT PCR. Results: SE was isolated from 16 of 84 samples from Set 1 and from 7 of 208 samples from Set 2 and 3. In Set 1, agreement between culture and RT PCR was 73.8%, 78.6%, 78.6%, and 76.2% for individual, pool of 2, pool of 3, and pool of 4 samples, respectively. Likewise, agreement for Set 2 and 3 was 93.8%, 97.1%, 95.7%, and 98.1%. Agreement with RT PCR for Set 2 and 3 at $Ct \leq 36$ was 99.0% (individual samples), 98.1% (pools of 2), 97.1% (pools of 3), and 98.1% (pools of 4). At $Ct \leq 30$, agreement was 100%, 99.0%, 100%, and 100% respectively. All samples at $Ct \leq 28$ had 100% agreement across the pools of two, three, and four. Conclusions: The increased agreement of Sets 2 and 3 over Set 1 is believed to be due to use of tetrathionate instead of buffered peptone water for RT PCR testing. The buffered peptone water pre-enrichment was likely unable to amplify SE growth to easily detectable levels. Pooling of environmental samples after enrichment does not impact sensitivity when compared to testing individual samples by culture or RT PCR.

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Genetic relatedness of *Salmonella* recovered from Michigan dairy farms in 2000 and 2009.
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Dairy farms serve as important reservoirs of *Salmonella* foodborne illnesses in the United States. Prior cross-sectional studies of *Salmonella* on dairy farms suggest important changes within this microbial population. Our objective was to use a longitudinal methods and molecular techniques to determine the genetic relatedness of *Salmonella* recovered from the same dairy farms between 2000 and 2009. We used a retro-prospective study design, serotype identification, and pulsed-field gel electrophoresis (PFGE) to test the hypothesis that sub-populations of *Salmonella* serotypes are genetically distinct between sources within farms, between dairy farms, and between years. Retrospective data included *Salmonella* recovered from six Michigan dairy farms in 2000. Comparable prospective data were collected from the same farms in 2009. Serotypes and genetic subtypes were identified using the Kauffman-White scheme and PFGE, respectively. The dice coefficient of similarity and cluster analysis (unweighted pair group method with arithmetic mean) were used to visualize the relatedness of isolates. Permutation testing was applied to compare the mean genetic similarity across comparison groups of interest. Within farms, there was no evidence of sub-populations of serotypes between samples from cows and calves, animal and environmental samples, or different classes of adult cattle ($p > 0.05$). Groups of isolates from different farms but within the same year were most frequently distinct ($p < 0.05$), but highly similar (>80% similarity). Serotypes recovered in both years were highly related, and two farms had indistinguishable isolates at each time point, suggesting long-term persistence or reacquisition of the same subtype. Serotypes recovered in both years but from different farms were distinct, but also highly related (>90% similarity). Overall, the relatedness of serotypes recovered 10 years apart was high, and comparable to the relatedness of serotypes recovered from different farms within the same year. Multi-locus sequence typing will be applied to these isolates to determine changes in evolutionary lineages, and the results will be presented.

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Salmonella in lymph nodes of cattle presented for harvest.

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Purpose: This study was undertaken to determine the prevalence of *Salmonella* in the subiliac lymph nodes (LN) of cattle.
Methods: LN samples were obtained from carcasses of cull and feedlot cattle at commercial packing plants. LN were trimmed of all fat, surface sterilized by submersion in boiling water for 3 seconds, placed in individual whirl-pak bags and then pulverized with a rubber mallet. Samples were enriched with tryptic soy broth and enrichments were subjected to immunomagnetic separation (IMS). IMS beads were transferred to Rappaport-Vasiliadis broth for secondary enrichment and *Salmonella* present were detected by culture on brilliant green sulfa and xylose lysine desoxycholate agars. Presumptive *Salmonella* isolates were confirmed by detection of *invA* by PCR, serotyped and their susceptibility to a panel of 15 antimicrobials determined.
Results: Between the months of September 2010 and July 2011, the mean prevalence of *Salmonella* in cattle subiliac LN (n = 2,564) was 5.3% (95% CI 1.3 - 8.8%). A seasonal and regional variation was observed with a *Salmonella* prevalence of 15.3% (95% CI 4.6 - 25.97) in the fall season from LN obtained from the Southern High Plains. LN collected in the summer months demonstrated a seasonal increase in prevalence among samples originating in the Midwest and Southern High Plains. The majority of *Salmonella* isolated were serotypes Montevideo and Anatum. Enumeration of *Salmonella* from positive LN (n = 25) showed the geometric mean CFU/LN to be 2.9×10^3 (95% CI 8.5×10^2 - 9.7×10^3). The majority of *Salmonella* were pansusceptible (84.3%); however, tetracycline resistance and the MDR-AmpC phenotype were occasionally observed.
Conclusions: These data demonstrate that *Salmonella* can readily be recovered from subiliac LN and consequently, beef trim containing LN may be a point-source for *Salmonella* entry into ground beef products. Moreover, harborage in LN protects *Salmonella* from the usual decontamination interventions used in packing plants. The public-health consequence of these findings needs to be investigated. Additionally, research is needed to better understand the opportunities to mitigate the risk of *Salmonella* harborage in LN of healthy cattle.

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Stress-adaptation can influence virulence in *Campylobacter jejuni*.

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Campylobacter jejuni is one of the leading causes of bacterial gastroenteritis. *C. jejuni* is a fragile bacterium requiring special conditions in the laboratory for culturing, but in nature this organism is able to survive in very diverse and hostile environments and produce disease in humans. The different mechanisms by which *C. jejuni* survives stressful conditions in the environment still remain unclear. Adhesion and invasion are thought to be important factors for the colonization of *C. jejuni* in the intestinal tract of host. Previous research in our laboratory showed that *C. jejuni* has the ability to induce an adaptive tolerance response to stresses like acid and aerobic conditions. In our research, different human and poultry isolates of *C. jejuni* were exposed to stresses including acid and starvation and the effects of these stresses on the adhesion and invasion abilities were evaluated in vitro using an INT 407 cell line. The exposure of acid-adapted *C. jejuni* ATCC strain 81176 to further stress of starvation for 24 h showed increased adhesion and invasion at 2h post-starvation. Starvation-adapted cells did not show any significant difference in their adhesion and invasion when exposed to further acid stress as compared to non-starved cells. Acid-adapted *C. jejuni* at pH 5.5, however, showed an increase in adhesion and invasion when exposed to a pH of 4.5 compared to non-acid-adapted *C. jejuni*. The degree of adhesion and invasion varied with strains of *C. jejuni* as well as with the time of adaptation to the mild stress. These data indicate that some stresses may increase the virulence of the organism. Studies involving expression of virulence genes and stress-adaptation are now underway.

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The relationship between the occurrence of *Campylobacter* in post-chill carcasses and flock prevalence at various sampling points in broiler production and processing.

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Purpose: *Campylobacter* is a leading cause of food-borne illness in the United States and poultry has been identified as a major reservoir. The purpose of this study was to determine the grow-out and processing plant sample(s) that best predict the likelihood of *Campylobacter* presence on broilers at post-chill.
Methods: Two broiler companies located in the Southern United States participated in the study. Within the two companies, 64 flocks from 32 farms from 10 complexes were sampled. Upon the day of chick placement into the grow-out house the gastrointestinal tracts of 30 chicks and 30 tray pads were collected from each flock. Prior to transportation to the processing plant and upon arrival at the processing plant, 30 ceca, crop, and whole bird carcass rinses were collected. During processing, 30 carcass rinses were collected prior to entering the immersion chill tank and again after exiting the immersion chill tank. Random-effects logistic regression was used to model the relationship between *Campylobacter* occurrence at post-chill and flock prevalence of *Campylobacter* at preceding segments. A univariate analysis was performed for selection of variables to include in a multivariable model.
Results: *Campylobacter* was isolated from 10.3% (209/2031) of post-chill carcass rinses. The univariate analysis showed all previous sample segments were significantly associated with occurrence of *Campylobacter* in post-chill carcass rinses.
Conclusions: The multivariable model revealed the best predictors of post chill *Campylobacter* carcass status were the whole bird carcass rinse in the grow-out house prior to transportation and the ceca upon arrival at the processing plant.

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Swine MRSA isolates form robust biofilms.

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Purpose: Methicillin-resistant *Staphylococcus aureus* (MRSA) colonization of livestock animals is common and prevalence rates for pigs have been reported to be as high as 49%. Measures to prevent, control, or eliminate MRSA in swine is of considerable public health concern. Bacterial colonization of both biological and non-biological surfaces followed by survival or persistence is often linked to the development of attached microbial communities known as biofilms. One hypothesis to explain high prevalence of MRSA in swine herds is the ability of these organisms to exist as biofilms.
Methods: To investigate the ability of MRSA swine isolates to form biofilms, a microtiter crystal violet assay was used to quantify biofilm formation by several swine and human isolates, including USA300. The contribution of known biofilm matrix components, polysaccharides, proteins and extracellular DNA (eDNA), was tested in all strains as well.
Results: All MRSA swine isolates formed robust biofilms similarly to human clinical isolates, including USA300. The addition of Dispersin B had no

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inhibitory effect on swine MRSA isolates when added at the initiation of biofilm growth and after pre-established mature biofilms formed. In contrast, the addition of proteinase K inhibited biofilm formation in all strains when added at the initiation of biofilm growth and after pre-established mature biofilms formed. Addition of DNase I at the initiation of biofilm growth inhibited biofilm formation in all strains, albeit with varied degrees of reduction. DNase I treatment of pre-established mature biofilms failed to disrupt biofilm biomass in all swine MRSA isolates.
Conclusions: In conclusion, swine MRSA isolates form robust biofilms and the biofilm matrix produced by these isolates is significantly composed of proteins and not polysaccharides. Additionally, eDNA is a component of the biofilm matrix, however the contribution of eDNA remains unclear given that not all pre-established mature biofilms produced by swine MRSA isolates were disrupted after DNase I treatment. Collectively, these findings provide a critical first step in designing strategies to control or eliminate MRSA in swine herds.

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Longitudinal study of veterinary students for acquisition of methicillin-resistant *Staphylococcus aureus* associated with exposure to pork production facilities. **A. Beahm**, J. Kinyon, L. Layman, L. Karriker, A. Ramirez, T. Frana;
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Introduction: Livestock-associated methicillin-resistant *S. aureus* (LA-MRSA) has been documented in U.S. swine and swine workers. Transmission studies have primarily focused on long-term exposure to livestock. This study focuses on short-term exposures that veterinary students encounter during visits to pork production facilities on their senior year rotation. Purpose: 1) Determine the rate of MRSA acquisition and longevity of carriage in uncolonized students exposed to pork production facilities during a two week clinical rotation, and 2) characterize the MRSA isolates from pork production facilities and assess their relatedness to human isolates. Methods: Student nasal swabs were collected at start of the rotation, pre- and post- visits to pork production facilities, non-visit days, and after completion of swine clinical rotation. Five pig nasal swabs and 5 environmental samples were collected during each visit to pork production facilities. Samples were processed with enrichment in broth, streaked onto chromogenic media and incubated for 24-48 hrs at 35º C. Suspect colonies were further characterized with biochemical testing, oxacillin screen with disc diffusion, PBP 2a latex testing, *mec*A and PVL PCR, and spa typing. Results: Thirty (30) veterinary students were enrolled in the study from May-November 2010. Forty (40) pork production facilities were visited during the same period. MRSA was detected in 30% (12/40) of pork facilities and appeared to significantly cluster within particular production flows. MRSA was detected in 22% (6/27) of students following exposure to a MRSA positive pork facility. Students found to be MRSA positive following a visit to MRSA positive pork facility were negative for MRSA within 24 hours and remained negative throughout the remaining sampling period. Spa types found in pork facilities (t002, t034, t548) closely matched those recovered from students (t002, t034, t548, t1107, t126) with few exceptions. Conclusions: Based on *spa* typing, not all MRSA recovered from pork production facilities can be considered LA-MRSA. The duration of MRSA carriage in students was brief and most likely represents contamination rather than colonization.

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Identification of PPK-1 and PPK-2 dependent transcriptome responses in *Campylobacter jejuni*.
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Purpose: Despite several years of research, *Campylobacter jejuni* continues to be one of the predominant causes of Gastrointestinal and food poisoning in the US and other developed countries. As a foodborne pathogen, *C. jejuni* is exposed to a variety of unfavorable environments during its infection cycle, both inside and outside the host. Despite the absence of classical stress response mechanisms, *C. jejuni* can successfully survive and be transmitted among susceptible hosts. Inorganic polyphosphate (poly P) and its associated enzymes; polyphosphate kinases (PPK1 and PPK2) provide alternative mechanisms for *C. jejuni* to mediate stress responses and pathogenesis; however, the precise molecular mechanisms are less understood. Here, we identified the PPK1 and PPK2-dependent genes by whole transcriptome analysis to address the global regulatory roles of PPK1 and PPK2.
Methods: Enriched mRNA from the purified total RNA of the parental strain and deletion mutants of *ppk1*, and *ppk2* from the log and stationary phase cultures was used to prepare an RNA-seq library. The libraries were barcoded with separate index oligos for Illumina sequencing.
Results: Sequence analysis indicated more than 60 genes to be differentially expressed in *Δ ppk1* mutant both, in the log as well as stationary phases. Similarly, the *Δ ppk2* mutant also had number of genes differentially expressed in the log and stationary phases.
Conclusions: These results support that both PPK1 and PPK2 serve as global regulators of stress responses and metabolism-related genes in *C. jejuni*.

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Loop mediated isothermal amplification method for detection of *Lawsonia intracellularis*.
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Purpose: *Lawsonia intracellularis* is the causative agent of proliferative enteropathy and is categorized as an economically important pathogen of swine and an emerging threat to the equine industry. Current methods of antemortem diagnosis include molecular tests such as polymerase chain reaction (PCR) or real time PCR, for the detection of *L. intracellularis* in fecal and tissue samples and serological tests for the detection of *Lawsonia* specific antibodies. Here we report on the development of a new diagnostic method, based on loop mediated isothermal amplification (LAMP), for the detection of *L. intracellularis* DNA in fecal samples.
Methods: For this, four sets of primers were designed and temperature conditions for the reaction were optimized.
Results: Using this method we were able to detect 2×10^4 mL^{-1} of *L. intracellularis* grown in cell culture. No amplification was obtained with DNA extracts from other swine pathogens, indicating high specificity of this new method. Furthermore, this method was used for detection of *L. intracellularis* in fecal samples without any extensive DNA extraction process or the use of thermocyclers.
Conclusions: Results of this preliminary study suggest that this method may be useful for detection of *L. intracellularis* in fecal samples when PCR testing is not readily available. Further advantages of this method include the fact that it is rapid and easy to perform, not inhibited by PCR inhibitors, and is cost effective. Further optimization studies are in progress.

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Lawsonia intracellularis increases *Salmonella enterica* levels in the intestines of pigs.

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Purpose: *Salmonella enterica* is a leading cause of food borne illnesses. It is our hypothesis that animal disease has the potential to dramatically increase food-borne pathogen risks to human health through an increase in pathogen loads on carcasses from diseased animals or herds. In support of this hypothesis, a recent prospective cross-sectional study of French pork production farms found a statistically significant association between *Lawsonia intracellularis* and carriage of S. enterica.
Methods: In the current study we measured *S. enterica* loads in 4 intestinal sites of pigs challenged with *L. intracellularis* (at 5 weeks of age), *S. enterica* serovar *Typhimurium* (at 6 weeks of age), or both microbes. At 7, 9, and 11 weeks of age two pigs per group were euthanatized and Salmonella and Lawsonia loads in jejunum, ileum, cecum, and colon were determined.
Results: The loads of *L. intracellularis* did not differ when co-challenged with *S. enterica* at any of the sites sampled. However, pigs challenged with both microbes had significantly higher levels of *S. enterica* in cecum and colon samples compared to pigs challenged only with *S. enterica* regardless of the time of sampling. At 11 weeks of age, the level of *S. enterica* in co-challenged pigs was approximately 105 per gram of tissue while the level dropped to approximately 101 per gram of tissue in pigs challenged only with *S. enterica*.
Conclusions: These results support our hypothesis that infections with *L. intracellularis* can adversely affect the carriage and shedding of food borne pathogens such as *S. enterica* and suggest that animal health can be linked to public health consequences.

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Mucosal immune system development in the small intestine of the newborn calf: regional differences in innate and acquired immunity.

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Mucosal dendritic cell (DC) development in the newborn is poorly understood despite evidence that distinct DC subpopulations populate individual mucosal surfaces. DC phenotype in the small intestine of newborn and weaned calves was analyzed using flow cytometry and DC distribution investigated with immunohistochemistry (IHC). Purification of $CD11c^{Hi}$MHC Class II^+ cells confirmed CD11c defined myeloid cells and analyzing neonatal blood and intestine revealed distinct mucosal DC subpopulations. $CD11c^{Hi}CD14^+$ cells were significantly more abundant in newborn ileum versus jejunum and $CD335^+$ NK cells were the only lymphoid population significantly different in ileum versus jejunum. IHC revealed unique patterns of myeloid cell distribution within the mucosal epithelium, lamina propria, and submucosa. $CD11c^+$ cells were present within the jejunal but absent from the ileal intraepithelial compartment. In contrast, $CD11b^+$ cells were present within the ileal but absent from the jejunal intraepithelial compartment. Significant age-related changes were apparent when comparing frequency and abundance of mucosal leukocyte subpopulations in newborn and weaned calves. Total mucosal leukocytes ($CD45^+$) increased significantly with age with the increase due to mucosal T cells. In particular, CD4 T cells and NK cells increased significantly in the jejunum and CD8, and [[Unsupported Character - ]]γδTcR T cells increased significantly with age throughout the small intestine. In contrast, $CD11c^{Hi}$MHC Class II^+ myeloid cells remained numerically unchanged with age but DCs ($CD13^+$, $CD26^+$, $CD205^+$) were enriched and macrophages ($CD14^+$,$CD172a^+$) depleted in older animals. Therefore, regional differences between ileal and jejunal mucosal leukocytes changed with age and there was also a significant age-dependent change in mucosal myeloid cell composition and distribution. In conclusion, the neonatal small intestine is populated by diverse myeloid subpopulations and significant differences in regional distribution are established early in life. These observations have significant implications for the response of the newborn to both commensal microflora and enteric pathogens.

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Transcriptional profiling of a pathogenic and an attenuated homologous Lawsonia intracellularis isolate during *in vitro* infection.

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Lawsonia intracellularis is the causative agent of proliferative enteropathy, an endemic disease in pigs and an emerging concern in horses. Spontaneous attenuated isolates obtained through multiple passages in cell culture do not induce disease. Conversely, bacterial isolates at low cell passage induce clinical and pathological changes. The identification of genes differentially expressed between a pathogenic and an attenuated homologous L. intracellularis isolate can help to elucidate virulence factor-encoding genes involved in this infection. The current study used high-throughput sequencing technology to characterize the transcriptional profiling of a pathogenic and an attenuated isolate during *in vitro* infection. Bacterial RNA was harvested from infected intestinal piglet epithelial cells (IPEC-J2) five days post-infection (approaching peak of infection). A total of 306 protein-encoding genes were expressed in both pathogenic and attenuated isolates. These common genes are involved in metabolic, biosynthetic and cell motility pathways, such as chemotaxis and flagellar assembly. Only 11 genes in the chromosome and one in the plasmid LIB (parA - ATPase involved in chromosome partitioning) were uniquely expressed by the attenuated strain. In contrast, 411 mapped genes were exclusive to the pathogenic strain. Genes involved in ATP-binding cassette transporter synthesis, two-component systems and protein export pathways were responsible for this wider transcriptional landscape and were distributed in the chromosome and three plasmids (LIA, LIB, LIC). In addition, only sequences from the pathogenic strain could be mapped onto the plasmid LIA, which may play an important role in the course of infection. We identified distinct genes and pathways between a pathogenic and an attenuated L. intracellularis isolate. This information supports our hypothesis and opens a new research field for studying target genes involved in the ecology, pathogenesis and physiology of L. intracellularis.

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A novel circular DNA virus from bovine stool is similar to chimpanzee stool-associated circular DNA virus, suggesting a new genus of circular DNA viruses.

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Purpose: This study was to characterize a novel circular DNA
virus identified from bovine stool.
Methods: A full genome of the novel circular DNA virus was obtained by PAN-PCR and Inverse PCR methods. The sequence analyses were done by CAP assembly, GLIMMER, COBALT alignment tool, Mfold and MEGA 4.0 programs.
Results: The virus named as bovine stool-associated circular DNA virus (BoSCV) had 2,600bp of genome size and two predicted major ORFs encoding relicase and casid proteins, which were inversely arranged on the genome. The stem-loop structure was located between 3'ends of two ORFs like chimpanzee stool-associated circular virus (ChimpSCV) rather than the other single circular DNA viruses, such as circovirus, cyclovirus, gyrovirus,

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nanoviridae and geminiviridae. BoSCV was also genetically closed to ChimpSCV with around 30% of identities in relicase and capsid proteins. In the phylogenetic analysis based on replicase protein, BoSCV and ChimpSCV were included in the same clade but differed from the other circular DNA viruses. In the field survey using BoSCV-specific PCR targeting ORF1, BoSCV and BoSCV-like sequences from bovine and porcine stools could be also detected and their sequences were closely related as a new group of viruses.
Conclusions: Although it's not clear whether BoSCV and ChimpSCV could infect hosts or not, they could be a new genus of the circular DNA viruses based on the genomic analysis.

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Quantitative evaluation of changes in C-reactive protein level and *Salmonella enterica* status as indicators of the swine health status in response to use of antibiotic growth promoter, Tylosin.
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Purpose: Antibiotics as growth promoters (AGPs) in agricultural animal production have been used for about 50 years. One suggested effect of the AGPs is that AGPs enhance the reduction in opportunistic pathogens and subclinical infections. Consequently this brings beneficial effects to animal health. The objective of this study was to evaluate the relationship between the animal health status, carriage of *Salmonella* and the use of AGP, Tylosin. Methods: Four groups of pigs (15pigs/group) in a commercial herd were used in our cross over study. Groups #1 and #2 were fed Tylosin beginning at 10 weeks of age at 40ppm, while groups #3 and #4 were fed feed without Tylosin. At 16 weeks of age, pigs in group #1 were removed from Tylosin, and pigs in group #3 began to receive Tylosin. This protocol was repeated in a second herd. Fecal samples from individual pigs were collected five times at 3-week intervals, and the quantity of *Salmonella enterica* was measured by using a most probable number (MPN) analysis. *Salmonella* isolates were subtyped using pulse field gel electrophoresis, and serotyped at the National Veterinary Services Laboratories. Four pigs from each group were bled at the same time points and C-reactive protein (CRP) level in serum measured using a commercial ELISA kit. Statistical analysis was conducted by using SAS v.9.2. Results: The level of CRP in each pig decreased as pigs grew. However, there was no significant correlation identified between the levels of CRP and treatment group. MPN and the number of pigs shedding *Salmonella* in each group also decreased as pigs aged, but there was no correlation between treatments and *Salmonella* status. Three *Salmonella* serotypes were identified in trial 1 and 2 serotypes in trial 2. These serotypes were detected throughout the experimental period.
Conclusions: Overall, this study showed that Tylosin did not affect changes of CRP levels, or load and prevalence of S*almonella* in pigs.

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Does infection caused by a multidrug resistant organism influence antimicrobial use practices in equine colic patients that had surgery?
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Purpose: To determine whether a nosocomial disease outbreak caused by a multidrug resistant *Salmonella* serovar (*S*. Newport MDR-AmpC) influenced antimicrobial drug (AMD) use in horses having colic surgery.
Methods: A retrospective study of horses undergoing colic surgery. Horses given pre-admission AMD, or presenting with co-morbidities that might affect AMD use were excluded. Pre- and postoperative AMD dose, pre-surgery timing, AMD duration (perioperative and total), number of AMD classes used and postoperative complications (thrombophlebitis, fever, incisional infection) were recorded. Surgical lesions were graded. Data were categorized by surgery date as pre- (01/01/01-12/31/03) or post-outbreak (11/01/04-12/31/07). Descriptive statistics were used to characterize patient populations. Associations between surgery date and categorical explanatory variables were determined by Fisher's exact test and quantified using logistic regression. Linear regression, with bootstrapping for 95% CI, was used to quantify relationships between surgery date and continuous temporal variables.
Results: There were 761 horses, 73 were euthanized under anesthesia due to poor prognosis. Of the remaining 688, 327 (47.5%) were treated pre- and 361 (52.5%) post-outbreak. There were no significant differences in age, gender, breed, or severity of lesion between groups. Nor was there any difference in time from pre-operative AMD administration to first surgical incision but in neither group did timing satisfy current guidelines. The number of different AMD classes used pre- and post-outbreak was the same, but AMD class changes were less common in post-outbreak patients (55% changed pre, 18% post, $P < 0.001$). Median perioperative (4 vs. 2.5, $P < 0.001$) and total (4 vs. 3, $P = 0.002$) days of AMD treatment were significantly lower post-outbreak. There were no differences in postoperative complications or discharge status between groups but days of hospitalization were significantly lower post-outbreak (8.6 vs. 6.8, $P < 0.001$).
Conclusions: A disease outbreak caused by a MDR organism can influence AMD prescribing practices. How long any such changes are sustained remains in question.

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A specific CpG site demethylation in the IFN-gamma gene promoter region of different aged equine.
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Advanced age in horses is associated with the increased expression of inflammatory mediators, including interferon-gamma (IFNγ). This "inflamm-aging" occurs in aged individuals from a variety of species, including humans. While the underlying mechanism responsible for this elevated inflammatory state is unknown, increased gene expression of pro-inflammatory cytokines appears to play a central role. Epigenetic modification of promoter elements can alter patterns of gene expression. These modifications can include alterations in histone acetylation and DNA methylation. Here we have examined the methylation status of specific CpG sites in the IFNγ promoter region of aged horses and compared it those of younger horses. We also compared DNA methyltransferase activity between the young and aged horses. Nine foals (<30 days of age) and 6 old horses (>20 years of age) were included in this study. Flow cytometry was used to obtain purified CD4+ T cells from peripheral blood mononucleocytes (PBMC) for DNA isolation and characterization. The DNA methylation status of specific CpG sites within the ifng promoter region was determined using methylation specific realtime PCR. DNA methylasetransferase activity was measured using an ELISA. Methylation of CpG within the IFNγ promoter region were significantly decreased in the old horses compared to the foals. There was correspondingly higher methyltransferase activity in the foals' PBMC compared to the older horses. Epigenetic modification of the promoter regions of aged horses could contribute to their increased expression of pro-inflammatory cytokines. DNA demethylation could play an important role in the inflammatory response correlates with aging.

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The DNA promoter of the interferon gamma gene (Ifng) is hypermethylated in neonatal foals.

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While interferon gamma (IFNγ) plays an important role in protection against viral and intracellular bacterial infections, its production in neonates is deficient. The deficiency is considered to be associated with neonatal susceptibility to various diseases. The underling mechanism for this deficiency remains unknown. Epigenetic modification of promoter elements can alter patterns of gene expression. Recent research found that the DNA around Ifng promoter is hypermethylated in human neonates and is associated with decreased IFNγ expression. Therefore, we hypothesized that the Ifng promoter in neonatal foals would also be hypermethylated, compared to older horses. Peripheral blood mononuclear cells (PBMCs) were isolated from both neonatal foals (< 7 days) and old horses (>20yrs) and CD4+ T cells were sorted. The methylation status of the three CpG sites on the Ifng promoter locus in CD4+ T cells from both foals and old horses was compared using methylation-specific PCR (MSP). The DNA methyltransferase (DNMT) activity associated with nuclear protein was also assessed using an ELISA method. Two out of the three CpG sites in the Ifng promoter region were found to be hypermethlyated in foals, though the DNMT activity was also lower in foals compared with old horses. In conclusion, the Ifng promoter region of neonatal foals is hypermethylated and could account for their decreased IFNγ expression.

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The effect of flunixin meglumine on the equine immune response to vaccination.

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Purpose: Horses are routinely vaccinated in order to prevent infectious diseases. While equine vaccines are safe and effective, adverse reactions to vaccines can occur. Non-steroidal anti-inflammatory drugs (NSAIDS) are sometimes used to decrease the incidence and severity of local vaccine reactions. However, there may be negative consequences of this practice in terms of the immune response to the vaccine. While NSAIDS have been shown to alter B cell responses in mice, their effect on the equine immune response to vaccination has not been examined.
Methods: Eighteen horses were randomly assigned to either a control (no NSAID) or treatment group (flunixin meglumine, 1mg/kg, PO BID x3d). All horses were vaccinated with a commercial, killed vaccine for equine influenza (EIV) and equine herpesviruses (EHV) 1 & 4. NSAID treatment commenced at the time of vaccination. Serum samples were analyzed for antibodies to EIV using HI and ELISA assays. Antibodies to EHV-1 were measured using an ELISA. Peripheral blood samples were also stimulated in vitro with both EIV and EHV-1 and then processed for RT-PCR analysis of IFN-γ, granzyme-B, IL-10, IL-13 and IL-2.
Results: The use of the NSAID resulted in a significant decrease in HI antibodies. There was also a significant decrease in IgG(T) antibodies to EIV in the NSAID group when compared with controls. Similarly, there was a significant effect of NSAID treatment on the total IG response to EHV-1. While NSAID treatment also significantly decreased EIV-specific cytokine mRNA production, there was no effect on the cytokine response to EHV-1.
Conclusion: Our results indicate that concurrent administration of NSAIDs when vaccinating horses can negatively impact the immunological response to the vaccine. Relative differences in the susceptibility of the vaccine antigens to the NSAIDS likely reflected prior immunological status to the antigen.

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The maturation of equine infectious anemia virus (EIAV) envelope-specific immune responses in vivo after exposure to a live-attenuated vaccine.

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Purpose: Distinct from other lentiviral infections, EIAV-infected horses will eventually enter an inapparent carrier state in which virus replication is tightly controlled by the immune system. Vaccination of ponies with an attenuated EIAV vaccine confers protection from disease. Previously, we have shown that the EIAV envelope (Env) protein is the primary determinant of vaccine efficacy. We hypothesize that Env specific T cell recognition will shift from immunodominant variable to conserved immunorecessive determinants in vaccinated ponies.
Methods: To test this hypothesis, sixteen ponies were vaccinated with the attenuated EIAV mutant and Env-specific immune responses were determined at monthly intervals post vaccination. Ponies were intradermally injected with peptide pools and individual peptides representing the Env protein. Punch biopsies were collected from the injection sites and paraffin-embedded for immunohistochemistry (IHC) detection of $CD3^+$ cells. Total RNA was isolated from biopsies for the determination of gene expressions of cell surface markers, CTL markers and cytokines by real-time PCR.
Results: One month post vaccination, the dominant immune responses were directed against those peptides in the carboxyl-terminal variable region. By contrast, six months post-vaccination the recognized peptides spanned the entire Env sequence, with a shift to the amino-terminal conserved region. There was a significant correlation between the percentage of $CD3^+$ cells detected by IHC staining and gene expression of CD8 and IL-2 in the biopsies. Those ponies recognizing the same specific peptides also shared at least one MHC I allele indicating that the recruited lymphocytes were likely antigen-specific, MHC-restricted $CD8^+$ effector or memory T cells
Conclusions: These results indicate that Env specific cellular immune responses are elicited by the attenuated EIAV vaccine and the shifting of peptide recognition from the variable region to the conserved region could explain the possible mechanism of protection afforded by this attenuated vaccine.

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T regulatory cells and IgE are inversely correlated in horses vaccinated with viral vaccines.

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Purpose: Type-1 hypersensitivities are IgE-mediated allergic responses that include severe systemic anaphylaxis. These reactions are caused by re-exposure to a specific antigen, to which the patient has previously been sensitized. During the last decade allergic responses to vaccination have shown a drastic increase. Our goal is to understand the etiology of Type 1 hypersensitivity reactions to vaccines in horses, and to develop an assay that would predict potential future reactors. We hypothesize that horses which are high IgE responders to BSA (a common contaminant in viral vaccines) will have low levels of specific T regulatory cells (previously known as T suppressor cells), and the inverse, i.e. high levels of T regulatory cells will be associated with low IgE responses. Since many viral vaccines share contaminating fetal bovine serum proteins as a potential antigen, we compared T regulatory cell production to IgE levels, both pre and post vaccination in adult horses. Methods: Sixteen adult horses ranging in age, were evaluated 1 hour prior to vaccination, and 7 days post vaccination for T regulatory cell production and specific IgE levels. Peripheral blood mononuclear cells (PBMC) isolated pre and post vaccination were stained and analyzed for extracellular expression of CD4 and CD25, and for intracellular Foxp3 using multicolor flow cytometry. BSA specific IgE was evaluated by ELISA. Additionally, a T regulatory cell recall response to BSA and FBS proteins was examined in these same horses by in

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vitro lymphocyte stimulation one-month post vaccination against different dilutions of BSA and FBS to re-stimulate a T-regulatory cell response. Results: Overall the percentage of T-regulatory cells increased significantly, while the level of IgE decreased or remained unchanged, from pre to post vaccination. Conclusion: there is an inverse correlation between the levels of T regulatory cells and the level of IgE responses to fetal bovine serum proteins in horses receiving viral vaccines.

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Deacylated polyethyleneimine and IL-15 expression constructs modulate humoral and cellular immune responses to DNA vaccination in horses.
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DNA vaccines are generally less effective in larger animals and elicit significantly weaker immune responses, than in small rodent model systems. To provide optimal protection against many pathogenic microorganisms, DNA vaccines must induce strong humoral and cellular immune responses. One limitation to DNA immunization in the horse is the difficulty in generating high levels of antigen-specific antibody and CTL-associated responses. Therefore, the goal of this study is to develop an improved DNA vaccine model system that is capable of stimulating robust immune responses to equine pathogens. The immunomodulatory effects of a deacylated cationic polymer, polyethyleneimine (PEI), and IL-15 expression constructs on equine immune responses to DNA vaccination were investigated using codon-optimized EIAV SU DNA (pSYNSU) as an antigen expression system. Four intradermal inoculations of SYNSU DNA complexed with PEI +/- IL-15 expression constructs were administered over five months. Increases in humoral immunity were assessed by IgG immunoblot analysis and IgGA ELISA. Lymphocyte proliferation and Real-Time RT-PCR assays were also performed to determine cellular increases to in vitro stimulation with SU-specific peptides. When compared to inoculations with just pSYNSU, administration of DNA vaccines formulated with PEI resulted in the production of very long-lived (15 months) humoral responses, and induced detectable cell-mediated IFN-y responses, following the fourth immunization. Additionally, the co-expression of IL-15 cytokines expanded the lymphoproliferative repertoire of T cell recognition to SU-specific peptides. DNA vaccination incorporating IL-15 (SSLSS) also significantly enhanced serum antibody levels of IgGA and IFN-y mRNA expression levels. It is evident from these vaccine studies that PEI enhances DNA vaccine-elicited antibody and CTL-associated responses in the horse and IL-15 (SSLSS) significantly augments these responses. These results demonstrate an important role for PEI and IL-15 in promoting the longevity of immune responses to genetic immunization, which has not been reported previously in any large animal model.

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Efficacy of attenuated Salmonella enterica serovar Typhimurium SA186, deleted of the zinc transporter ZnuABC, to control pig salmonellosis.
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Purpose: Salmonella enterica serovar Typhimurium has long been recognised as one of the most important zoonotic pathogens. We recently demonstrated that the attenuated strain Salmonella enterica serovar Typhimurium, unable to synthesize the zinc transporter ZnuABC (S.Typhimurium SA186), is able to induce a mild infection and to stimulate an immune-based protection against systemic and enteric salmonellosis in mice. These findings suggested that this attenuated mutant strain could represent an interesting candidate vaccine for mucosal delivery. The aim of this study was to assess the safety and the protective effect of this attenuated Salmonella strain in pigs.
Methods: We inoculated piglets and post weaned pigs by oral route with different doses of S.Typhimurium SA186 and then we challenged them with fully virulent S.Typhimurium.
Results: We showed that the clearance of the vaccine strain was correlated to the dose of vaccination, that the vaccine has a negligible effect upon performances, and after the challenge infection, we showed that S.Typhimurium SA186 can induce a good degree of protection with the reduction of the colonization of virulent strain either in the gut or in the systemic organs.
Conclusions: These studies demonstrate that S. Typhimurium SA186 is a promising vaccine candidate to protect pigs against S.Typhimurium infection and to reduce the shedding of virulent S.Typhimurium in the environment.

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Modulation of MHC I & II and cytokine expression by EHV-1 ORF 1/2 at the respiratory epithelium
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Purpose: Despite the importance of EHV-1, effective prevention remains elusive and is likely a result of immunomodulation by EHV-1 genes. Recent data has implicated the ORF1/2 genes as candidate immunomodulatory genes. Because the ORF1/2 genes are expressed shortly after infection and before the onset of adaptive immunity, it is likely that they target early innate responses of the respiratory epithelium. The purpose of our study was to determine the immunomodulatory effects of the EHV-1 ORF1/2 genes at the respiratory epithelium.
Methods: Differentiated equine respiratory epithelial cells (ERECs) were infected with EHV-1 strain Ab4 (WT), ORF1/2 and ORF1 deletion mutants or their revertants at a multiplicity of infection of 10. Cytokine mRNA responses were determined using quantitative real-time PCR. In addition, major histocompatibility complex (MHC)-I and MHC-II as well as toll-like receptor (TLR) 3 and TLR9 protein expression were examined using FACS analysis.
Results: Infection with WT virus down-modulated MHC I expression in infected cells, and MHC II in infected and uninfected neighboring cells. Deletion of the ORF1/2 genes or ORF1 gene alone partially reversed the MHC-I and MHC II downmodulation, while no differences in MHC expression of ERECs were observed following infection with the ORF1 or ORF1/2 revertants and WT virus. In addition, while infection increased TLR 3 and TLR9 expression in the infected cells, TLR expression was not increased in the uninfected neighboring cells and deletion of the ORF1 and 2 genes did not affect TLR expression. Finally, while WT infection increased expression of pro-inflammatory cytokines and chemokines, deletion of the ORF1/2 genes decreased IL-8, IL-1 and TNF-alpha mRNA responses when compared to WT infection of ERECs.
Conclusion: There is evidence that the ORF1/2 genes modulate expression of MHC-I and MHC-II, which are important molecules for antigen presentation and decrease induction of IL-1, TNF-alpha and IL-8. In the future we will further elucidate the target mechanisms leading to MHC I & II modulation and determine how these early events affect spread of the virus to underlying immune cells and ultimately disease pathogenesis.

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Differential regulation of mucosal immune responses at various mucosal tissues in pigs infected with *PRRSV* strain *VR2332*.

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Porcine Reproductive and Respiratory Syndrome has been devastating the swine industry economically for the past two decades. The RNA virus which primarily infects the alveolar macrophages of pigs occurs as two major prototype strains, VR2332 and the Lelystad virus. The VR2332 is the strain commonly occurring in North America and also forms the basis of the attenuated live vaccine available commercially. This study was conducted to understand the pathogenesis of VR2332 and its modulation of immune correlates at mucosal tissues. Pigs were infected with VR2332 virus intranasally and euthanized at post infection days (PID) 15, 30 and 60. Gross and microscopic examination of the lungs of infected pigs showed pneumonic lesions at PID 15 and 30, whereas virus titers remained high until PID60 in the lungs. Similarly, viremia was maintained in all the infected pigs until PID 60.The virus neutralizing antibodies started to appear from PID 7 and was maintained at low titers. Re-stimulatory cytokine analysis of immune cells detected a moderately increased Th1 cytokines (IFN-γ, IL-12) secretion and immunoregulatory (IL-10) and proinflammatory (IL-6) cytokines were high until PID 60. In serum, IFN-γsecretion was detected at PID35, while IL-10,IL-6, and TGF-β were maintained high throughout the study. Flow cytometric analysis of mononuclear cells from different sites (lungs, blood, tonsils, tracheobronchial and iliac lymph nodes) identified varying trends for total T Lymphocytes, cytotoxic T lymphocytes, memory cells, natural killer cells, and T regulatory cells in each tissue, which indicated overall immunopathogenesis of the virus. This information may further pave the way in formulation of preventive and therapeutic measures against PRRSV.This project was supported by National Pork Board, USDA-NIFA PRRS CAP2, and OARDCThe Ohio State Universityto RJG.

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Comparison of serological assays for *Actinobacillus pleuropneumoniae* (serotypes 1-9) on serum from pigs experimentally infected with APP or vaccinated with APP bacterins.

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Purpose: *Actinobacillus pleuropneumoniae* (APP) is found worldwide, is a major cause of bacterial pleuropneumonia in pigs, and is frequently associated with high death loss when introduced in naïve populations. There are 15 known serotypes ranging in pathogenicity from mild (serotype 3) to highly pathogenic (serotypes 1, 5, and 7). Several serological assays are currently available including three new serogroup specific ELISAs (1-2-9-11; 4-5-7, and 3-6-8-15) that were recently introduced into veterinary diagnostic laboratories. The objective of this study was to determine the diagnostic performance of the complement fixation (CF) assay compared to the three new ELISAs.
Methods: Serum samples tested included samples from pigs vaccinated intramuscularly with an inactivated APP bacterin (a bacterial culture grown in PPLO for 18 hours, inactivated, then 1.5ml of 10% aluminum hydroxide adjuvant added) or inoculated intranasally with live APP. This study was conducted in two parts: In part A, eight 8-week-old pigs were randomly divided into four groups of two pigs each and each group was vaccinated with inactivated APP serotype 1, 3, 5 or 7, respectively. In part B, eighteen 8-week-old pigs were randomly divided into nine groups of two pigs each and each group was inoculated with live APP serotype 1 through 9, respectively. In both parts, blood was collected weekly and serum tested by CF and ELISA for APP-specific antibodies.
Results: The results indicate that vaccination using inactivated bacterins did not result in a detectable IgG response in 62.5% (5/8) of the vaccinated pigs. Animals challenged intranasally with live APP developed anti-APP antibodies as early as 7 days post inoculation on both the ELISA and the CF assays. However, the ELISA was better at correctly identifying serotypes.
Conclusions: The new ELISAs appear to be slightly more sensitive than the CF assay for detecting anti-APP antibodies induced by killed bacterins or intranasal challenge.

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Early vaccination of 5-day-old piglets does not alter the efficacy of two commercial porcine circovirus type 2 vaccines in an experimental triple infection challenge model.

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Purpose: Since 2006 there has been a marked increase in use of porcine circovirus type (PCV2) vaccines by North American pork producers to manage porcine circovirus associated diseases (PCVAD). All commercial vaccines currently available in the US are licensed for use in pigs that are 21 days of age or older; however, many producers prefer to minimize handling of the pigs and use the vaccine between two and five days of age when young piglets are undergoing other procedures (castration, iron shots, tail docking, tooth clipping, etc.). The objective of this study was to compare the efficacy of PCV2 vaccination at 5 and 21 (d5 or d21) days of age using either a killed chimeric PCV2 vaccine or a subunit vaccine.
Methods: Forty-eight PCV2 naïve piglets were randomly separated into six groups and vaccinated at either d5 or d21 (positive control, negative control, Chimeric-d5, Chimeric-d21, Subunit-d5, and Subunit-d21). All groups except for the negative control were challenged concurrently with PCV2b, porcine parvovirus (PPV), and porcine reproductive and respiratory syndrome virus (PRRSV) on d49.
Results: By 14 days after vaccination, all vaccinated pigs had developed detectable anti-PCV2 antibodies. By the time of challenge at d49, the pigs vaccinated at d5 had significantly higher anti-PCV2 antibodies than the pigs vaccinated at d21 and both groups vaccinated with the chimeric vaccine had significantly higher levels of neutralizing antibodies than the groups vaccinated with the subunit vaccine. Both vaccines and vaccination regimens were efficacious as evidenced by significantly lower levels of PCV2 viremia and decreased prevalence and severity of PCV2-associated microscopic lesions in all vaccinated pigs compared to the pigs in the positive control group. Two of eight (25%) positive control pigs developed severe lymphoid lesions associated with abundant PCV2 antigen consistent with PCVAD.
Conclusions: This work provides evidence that vaccination of PCV2 naïve piglets at d5 in an experimental triple challenge model results in earlier development of anti-PCV2 antibodies and induces significant reduction or complete protection against PCV2 viremia and PCV2-associated lesions.

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Nanoparticles entrapped killed *PRRSV* vaccine reduces *PRRSV* viremia in both homologous and heterologous *PRRSV* challenged pigs.

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Porcine reproductive and respiratory syndrome remains as the leading cause of economic burden to US swine producers in spite of 20 years of research efforts to control the disease. Intranasal vaccination of killed PRRSV antigens marginally reduced the virus titer in blood to homologous (VR2332) as well as heterologous (MN184) viral challenge. To potentiate the effect of killed PRRSV vaccine (killed vaccine), poly (lactide-co-glycolide) (PLGA) - nanoparticles were prepared to encapsulate PPRSV killed antigens (Nano-PRRSV). Nano-PRRSV received pigs showed reduction in viremia on post-challenge day 8 and complete viral clearance by day 15 to both VR2332 and MN184 PRRSV challenge. Immunohistochemistry analysis showed more PRRSV antigens in the lungs of challenged pigs of unvaccinated and killed vaccine inoculated compared to Nano-PRRSV inoculated pigs. Hematoxylin &

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Eosin staining of the lung sections revealed severe infiltration of mononuclear cells in unvaccinated and killed-vaccine inoculated compared to Nano-PRRSV received virus challenged pigs. Immune responses based on the frequency of various cell populations (Natural Killer cells, T-helper, T-cytotoxic, Gamma-delta T cells and T-regulatory cells) at mucosal and systemic sites and the cytokine secretions, namely IFN-α (Innate), IL-12 & IFN-γ (Th1), and IL-6 (pro-inflammatory) in lungs, serum, and by re-stimulated immune cells supported our results. In addition, PRRSV specific IgA and IgG antibodies and virus neutralizing antibody titers were also detected in nasal wash, lung lysate and serum. Overall, our results suggested that intranasally administered PLGA-nanoparticles-PRRSV-Killed vaccine is capable of inducing protective immunity to PRRSV. This project is supported by National Pork Board to RJG.

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Immunology and Animal Health: the whole is greater than the sum of its parts.
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The ultimate goal for researchers attending CRWAD is improvement in animal health, to benefit both the animals themselves and the people who work or play with them, or consume animal products. My own research has focused principally on the study of immunity to infectious diseases in ruminants, in particular diseases associated with Pasteurellaceae infections of cattle and chlamydial infections in sheep. The goal has been to improve animal health through better management of these infections so that disease is averted. Early in my career, with colleague and mentor Bruce Wilkie, we developed the first of what are now the standard vaccines for bovine pneumonic pasteurellosis. This was achieved by examining *Mannheimia haemolytica* respiratory infection from an immunological perspective that emphasized the host:parasite interaction. This approach resulted in identification of several previously uncharacterized bacterial antigens and virulence factors, including leukotoxin, and development of a novel patented process for their incorporation in a vaccine. Subsequent longstanding collaboration with bacterial geneticist Reggie Lo, led to molecular characterization of leukotoxin and additional virulence factors, discovered primarily through examination of host response to naturally acquired infection, especially in animals that resisted pneumonia. The success of these endeavors reinforces the importance of a research approach that examines the interaction between the host and the pathogen, not either in isolation.

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The reduction of bacterial mastitis severity by treatment with 25-hydroxyvitamin D3.
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Purpose: Studies have shown that activated macrophages in the mammary gland convert 25-hydroxyvitamin D3 (25(OH)D3) to 1,25-dihydroxyvitamin D3 (1,25(OH)2D3), and that 1,25(OH)2D3 affects gene expression in immune cells. In fact, a deficiency in the serum levels of 25(OH)D3 has been correlated with increased risk of infectious diseases in humans. Milk typically has little 25(OH)D3 and therefore its addition into the mammary gland during an infection may have an affect in animals with sufficient serum levels of 25(OH)D3. In this study we wanted to determine the effect of infusing 25(OH)D3 on a mastitis infection.
Method: We infected 10 cows with Streptococcus uberis in one quarter. Five cows were infused subsequent to each milking after infection (twice daily) with 100ug of 25(OH)D3. The other 5 were infused with the control solution.
Results: There was a significant decrease of bacterial counts ($P<0.05$) of the 25(OH)D3 treatment. In addition, there were reductions ($P<0.07$) in rectal temperatures and levels of BSA in milk in the 25(OH)D3 treated animals compared to the controls. There was a significant ($P <0.05$) time x treatment effect, as the 25(OH)D3 treated animals milk production declined because peak inflammation occurred later in the infection compared to control cows.
Conclusions: The data gathered in this study demonstrates that in vivo administration of 25(OH)D3 used as a treatment, reduces the severity of an intramammary infection. The effectiveness of 25(OH)D3 may be due to many factors, including a predominant role of the innate immune response in mastitis and that the milk normally has low 25(OH)D3 levels.

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Mechanisms behind *Mycobacterium avium* subspecies paratuberculosis suppression of host cell apoptosis in primary bovine macrophages.
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Infection of ruminants with Mycobacterium avium subspecies paratuberculosis (MAP) causes a chronic inflammatory condition known as Johne's disease. This disease currently affects over 68% of American dairy farms and causes as much as $1.5 billion in losses each year to the dairy industry. Viable MAP bacteria have been detected in commercially available meat, milk, and cheese, a concern because MAP has been linked to the human inflammatory condition known as Crohn's disease. MAP is particularly hard to control because it is an intracellular organism and prevents macrophage phagosome maturation resulting in persistent infections. Previously, we found that MAP dramatically altered the transcriptome of infected macrophage cells in culture. Macrophage transcriptome profiles following infection with 10 different MAP strains isolated from four different species identified several pathways that are altered in MAP infected cells. Genes encoding regulators of apoptosis appeared to be preferentially affected by MAP. Utilizing a flow cyotmetric approach, we demonstrated that MAP-infected macrophages were significantly less likely to enter spontaneous apoptosis than either uninfected cells in the same culture (bystanders) or cells from cultures not exposed to MAP (control cells). MAP infection also hampered induction of apoptosis in macrophages following exposure to peroxide. In initial studies aimed at uncovering the mechanism behind MAP-induced resistance to apoptosis in macrophages, we have examined the activity of cellular caspases, major regulators of apoptosis in many cells. MAP infection appears to block activation of caspases in both spontaneous apoptosis and in cells exposed to peroxide. Reduced caspase activity in MAP infected cells may be due to a reduction in caspase mRNA abundance. MAP infection also reduces expression of proapoptotic proteins, such as BAD, and phospho-BAD. Our results offer one explanation for the dramatic build up of infected macrophages at sites of MAP infection in tissues, with infected cells failing to undergo apoptosis. This also may have a negative impact on development of MAP immunity by limiting antigen presentation following effercytosis.

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Bovine macrophages produce extracellular traps in response to Mannheimia haemolytica and its leukotoxin.
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Bovine respiratory disease (BRD) can lead to a pleuropneumonia that is characterized by intense inflammation, extensive neutrophil infiltration, fibrin deposition, and consolidation of the lungs. One can also detect extracellular DNA within the airways of cattle with BRD. One possible source of this DNA is from leukocytes that die, or release DNA to form a fibrillar network refereed to as extracellular traps. Previous research has shown that bovine neutrophils produce neutrophil extracellular traps (NETs) in response to *Mannheimia haemolytica* and its leukotoxin (LKT) in vitro. Because macrophages

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provide the first line of cellular defense in the lungs, we examined whether they too produce macrophage extracellular traps (METs) in response to *M. haemolytica* or LKT. We quantified a dose- and time-dependent increase in MET formation by bovine macrophages in response to either purified leukotoxin (LKT) or *M. haemolytica* cells *in vitro*. MET formation peaked at 5 minutes, was dependent on NADPH oxidase activity, and the DNA within METS appeared to be of nuclear rather than mitochondrial origin. Using confocal and scanning electron microscopy, we observed METs ensnaring *M. haemolytica* cells *in vitro*. Further investigation revealed that METs produced in response to LKT trapped and killed *M. haemolytica* cells more efficiently than METs formed in response to *M. haemolytica* cells alone. Murine and human macrophage cell lines also formed METs when they were stimulated with a related RTX toxin, the *E. coli* hemolysin. Our findings suggest that MET formation provides a second mechanism (in addition to phagocytosis) by which bovine macrophages can attack and kill *M. haemolytica*. These data suggest a possible role for MET formation in the host response to *M. haemolytica* infection in cattle.

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Impaired capacity of neutrophils to produce reactive oxygen species, release extracellular traps and express genes encoding for cytokines may contribute to altered immune function in periparturient dairy cows.
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Purpose:The objective of this study was to examine the function and gene expression of bovine neutrophils (PMNL) during the periparturient period.
Methods: PMNL were collected from the blood of cows (n = 20) on days 49, 28, 19, and 9 prepartum and 1, 7, 14, and 30 postpartum to determine their generation of reactive oxygen species (ROS), formation of extracellular traps (NETs), chemotaxis and killing of *Staphylococcus aureus*. To identify alterations in the expression of genes, mRNA was purified from PMNL collected from midlactation (146 d postpartum; n = 10) and early lactation cows (7 d postpartum; n = 10) after a 120-min incubation with 0 or 50 µg/mL of LPS. Amounts of *IL-8*, tumor necrosis-α (*TNFA*), bactericidal/permeability-increasing protein (*BPI*), myeloperoxidase (*MPO*), superoxide dismutase 2 (*SOD2*), NADPH oxidase 4 (*NOX4*) and cytochrome b-245, alpha polypeptide (*CYBA*) mRNA were determined relative to β-actin by real-time quantitative PCR.
Results: Production of ROS by PMNL increased 25% from days 49 to 19 prepartum and declined 50% to reach a lowest level on day 1 postpartum. ROS generation remained low on days 7 and 14 postpartum and recovered 30 days after parturition. NET release by PMNL was highest on day 49 prepartum, decreased 31% on day 28 prepartum, remained low throughout the periparturient period and slightly improved by day 30 postpartum. Regardless of stage of lactation, PMNL incubated with 50 µg/mL of LPS had 607 and 82% higher mRNA contents of IL-8 and SOD2 compared with 0 µg/mL LPS, respectively. Also, LPS augmented the expression of TNF, CYBA and BPI (2129, 18 and 72% compared with controls, respectively) only in PMNL from midlactation cows. Independent of LPS treatment, PMNL from midlactation cows had 61% higher mRNA contents of IL-8, compared with PMNL from early lactation cows. PMNL from early lactation had a 469% increase in MPO mRNA expression, relative to PMNL from midlactation cows.
Conclusions:These results suggest that impaired ROS production, release of NETs and lower expression of genes encoding for cytokines and enzymes involved in ROS production by PMNL contribute to the altered immune function observed in periparturient dairy cows.

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Selenoproteins alter eicosanoid biosynthesis in macrophages.
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Purpose: Selenium (Se), functioning primarily through selenoproteins, is an essential nutrient in the mammalian diet and sufficient intake is critical for optimum immune function. Deficient Se-nutrition is associated with the progression of several chronic, inflammatory-based diseases including mastitis, metritis, and sepsis in dairy cows as well as atherosclerosis in humans. Macrophages play an important role in the progression of inflammatory-based diseases, particularly through the synthesis of eicosanoids that can either promote or resolve inflammation. Therefore, this study addresses the hypothesis that reduced selenoprotein activity will enhance gene and protein expression of COX and LOX enzymes, and increase the biosynthesis of pro-inflammatory eicosanoids.
Methods: Both an *in vitro* RAW 264.7 macrophage cell line based on Se-status and an in vivo selenoprotein conditional knockout mouse model were used to modify selenoprotein status. Differences in reactive oxygen species (ROS) production between selenoprotein deficient and selenoprotein adequate macrophage populations was assessed using hydroxy-H_2DCFDA. Differences in pro-inflammatory gene and protein expression with respect to selenoprotein status were assessed by qPCR and Western blot, respectively. The impact of selenoprotein activity on eicosanoid production was measured by liquid chromatography- mass spectrometry (LC-MS).
Results & Conclusion: We showed that significant decreases in selenoprotein status resulted in accumulation of ROS and oxidative stress. Significant increases in COX1, COX2, and 15LOX gene and protein expression also were observed in selenoprotein-deficient macrophages. Studies are currently underway to profile the changes in eicosanoid production as a consequence of altered COX and LOX expression. Future studies will focus on determining the mechanisms by which selenoprotein status can influence macrophage pro-inflammatory phenotype through control of eicosanoid biosynthesis.

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Probing the effects of dual infections with *Mycobacterium avium* ss. *paratuberculosis* and bovine leukemia virus on Regulatory T cell prevalence and activity in cattle.
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Johne's disease (paratuberculosis) is a chronic wasting disease of wild and domestic ruminants caused by *Mycobacterium avium* subspecies *paratuberculosis* (MAP). Previously, we proposed that the chronic nature of Johne's disease may result in development of a regulatory T cell (Treg) population (characterized as CD4+/CD25+/FoxP3+ and by secretion of transforming growth factor beta (TGFb) or interleukin 10 (IL-10)) in the host. These cells function to shift the immune balance away from a pro-inflammatory Th1 response, necessary to combat intracellular infections, to an unproductive Th2 immune response. Bovine leukemia virus (BLV) is also an intracellular pathogen resulting in chronic infections of cattle, and like MAP, BLV infection is extremely prevalent in dairy cattle. Due to the high frequency of concurrent MAP and BLV infection, and the commonality of being chronic infections capable of eliciting Treg activity, the effects of BLV infection on Treg prevalence and function have also been probed. Methods including *in vitro* stimulations of peripheral blood mononuclear cells (PBMCs), PCR, histology, and real-time PCR are used to help address these aims. A combination of factors consisting of interleukin 2, rapamycin, and TGFb is shown to enhance the relative proportion of regulatory T cells in PBMC populations in a monocyte feeder-layer stimulation experiment. Early results from our lab demonstrate a reduction in Treg outgrowth in animals infected with BLV compared to BLV-free controls, and these results are supported by histological work showing substantially more Tregs in lymph nodes of BLV-negative cattle as compared to controls. Initial studies have also revealed substantial up-regulation of TGFb-2 transcript expression in lymphatic tissues of

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animals infected with MAP and BLV compared to healthy controls, while BLV infection alone results in a modest upregulation of IL-10 transcript expression. The cell source of TGFb-2 in dual infected animals has not yet been determined, but is being investigated. Our results suggest that while BLV may not, in isolation, produce significant losses, it may have a rather dramatic effect on how the host responds to other pathogens, such as MAP.

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Proinflammatory responses of bovine endothelial cells to non-esterified fatty acids.
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Purpose: A positive correlation exists between increasing concentrations of plasma non-esterified fatty acids (NEFA) in the prepartum period, disease susceptibility, and altered host inflammatory responses. The vascular endothelium regulates inflammation, in part, by eicosanoid biosynthesis. The vascular endothelium is exposed to NEFA during the peripartum period because of accelerated lipolysis at this time and these fatty acids can serve as substrates for eicosanoid biosynthesis. Changes in the composition and concentration of NEFA can affect eicosanoid biosynthesis. Hence, we created an in vitro model of the peripartum vascular endothelial environment and assessed eicosanoid biosynthesis associated with NEFA at various physiological doses. We hypothesize that NEFA will enhance bovine endothelial cells proinflammatory gene expression, protein synthesis, and eicosanoid biosynthesis in a dose dependent manner.
Methods: Bovine aortic endothelial cells were cultured for 24 hours in 10% fetal bovine serum media supplemented with albumin bound 0.25mM, 0.5mM, 0.75mM NEFA (3% C14:0, 30% C16:0, 45% C18:0, 16% C18:1, 5% C18:2, and 5% C22:6). A 12-hour 50ng/ml LPS challenge in NEFA free media served as positive control. NEFA free media with and without albumin served as negative controls. Gene expression was measured by qPCR, protein quantified by western blot, and eicosanoids quantified by LC-MS. Differences between NEFA concentrations and between NEFA concentrations and positive and negative controls were made with multiple t-tests with adjustment by Tukey's method. Significance was ascribed at $p \leq 0.05$.
Results: Gene expression of IL-6, IL-8, ICAM-1, VCAM-1, and COX-2 increased with NEFA dose, as did abundance of ICAM-1 and COX-2 protein and concentration of PGE_2, 9-HODE, and 13-HODE, suggesting an enhanced proinflammatory state.
Conclusion: NEFA enhance bovine endothelial cells proinflammatory gene expression, protein synthesis, and eicosanoid biosynthesis in a dose dependent manner. Current work is identifying the possible mechanisms of NEFA activation of gene transcription and NEFA influence on eicosanoid biosynthesis.

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Inhibition of Mycoplasma mycoides subsp. mycoides adherence to transformed bovine skin fibroblasts by Mycoplasma specific monoclonal antibodies.
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Contagious bovine pleuropneumonia (CBPP), a contagious respiratory disease of cattle is caused by Mycoplasma mycoides subsp. mycoides (Mmm). While the preferred disease control method is by vaccination, the available vaccines have low efficacy and numerous side effects. In this regard, detailed knowledge of the molecular mechanisms of pathogenicity of Mmm is a prerequisite to designing safe and efficient vaccines. Our hypothesis is that preventing Mycoplasma spp from binding to their host tissues will prevent colonization and thus prevent establishment of infection. Although several other Mycoplasma adhesins have been identified, similar molecules have not been detected for Mmm. This research was designed to assess the variation in adherence between different Mmm strains and cell lines; and the capacity to inhibit adherence by Mmm-specific monoclonal Antibodies (mAbs). Mmm strains were allowed to adhere to cells for 2hrs and stained with Mmm specific rabbit serum. Cells bound to Mycoplasma were measured by Flow Cytometry. There was variation in the binding capacity of the different Mmm strains tested. On average, 82% of the cells tested were fluorescent with the Mmm strain Afade while only 30% with T144, the vaccine strain. On the other hand, there was no significant difference in the binding rates between the 3 cell-lines tested with binding rates in the range of 55-60%. Of the 23 mAbs tested, 7/23 (30%) were able to inhibit >30% binding of Mmm to transformed skin fibroblasts. Antibody AMMY1 inhibited binding by 62.9%, AMMY3 by 40.4% and AMMY4 by 46.3%. Based on our preliminary data, cytadherance of Mmm strains appears not to be dependent on the cell line type ($p>0.05$) but rather on the strain itself ($p<0.05$). The ligand(s) identified by the mAb could possibly be responsible for binding of Mmm to receptors on bovine cells and therefore should be considered for further testing and their potential use as vaccine candidates.

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Attenuation and protective efficacy of live attenuated *Salmonella* Gallinarum vaccines by employing a regulated delayed attenuation strategy.
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Purpose: Fowl Typhoid (FT) is a systemic disease of chickens and turkeys, resulting in severe loss of poultry in the developing world. The causative organism of FT is *Salmonella* Gallinarum, a non-flagellated, host-restricted Group D *Salmonella*. The disease is manifested by an initial phase of acute diarrhea, followed by a severe infection in the systemic organs and subsequent morbidity or mortality or clearance. Currently, the live vaccine for FT, 9R, is available as an injectable vaccine for older birds. However, the strain is virulent in younger birds and its genetic alterations are not well defined, although it is known that the strain is permanently rough. To reduce costs associated with administering the vaccine and to enhance safety, we are developing a safe, orally administered vaccine that provides long-lasting immunity. Such a vaccine would be ideal for the developing world, where fowl typhoid is largely uncontrolled and the cost associated with the current vaccine is prohibitive.
Method: To evaluate candidate vaccines, we developed a challenge model for creating fowl typhoid via oral inoculation of 4-week old chickens with *S.* Gallinarum. We utilized a ten-point scoring system to assess the health of birds post-vaccination and post-challenge. To create vaccine candidates, we employed a regulated delayed attenuation strategy using conditional mutations affecting O-antigen synthesis. The resulting mutants, when grown in the presence of specific sugar/s *in vitro*, produce smooth, full-length LPS but become rough after several generations of growth *in vivo*.
Results: We found that a Δ*pmi* mutation alone or in combination with a ΔP_{rfc}::TT *araC* P_{BAD} *rfc* mutation is highly attenuated in young chicks and provides high levels of protection against a lethal oral dose of wild-type *S.* Gallinarum. A candidate mutant, ΔP_{crp} ::TT *araC* P_{BAD} *crp*, was also attenuated and provided protection against *S.* Gallinarum and cross-protection against colonization by the human pathogen *Salmonella* Enteritidis.
Conclusion: This work demonstrates the potential of developing an oral, live attenuated *Salmonella* Gallinarum vaccine using regulated delayed attenuation.

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Intranasal vaccination with Ad5-encoding influenza HA elicits sterilizing immunity to homologous challenge and partial protection to heterologous challenge in pigs.
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Vaccine availability during the 2009 H1N1 pandemic highlighted the lengthy production time of traditional vaccine. Replication defective adenovirus (Ad5) constructs with influenza genes have been investigated as candidate vaccines with rapid production potential. However, the primary model used for investigation of Ad5-influenza vaccines has focused on subcutaneous immunization in mice. We compared the efficacy of a single intranasal dose of an Ad5 vaccine to traditional intramuscular (IM) administration of an adjuvanted whole inactivated virus (WIV) vaccine following homologous and heterologous challenge in swine. Pigs were immunized with an Ad5 construct containing a codon optimized hemagglutinin gene (Ad5-HA) of A/CA/04/2009 (H1N1). Adjuvanted, WIV (A/CA/04/09) vaccine was given IM with a boost at day 21 post vaccination. Animals were challenged intranasally at 42 days post vaccination with A/CA/04/09 or A/swine/MN/02011/08 (MN, H1N2). The Ad5-HA vaccine provided sterilizing immunity to homologous challenge. Following heterologous challenge (MN) in Ad5-HA vaccinated pigs, macroscopic lung lesions were not different than non-vaccinated/challenged controls (NV/MN), but nasal virus shedding was reduced by 3 days post infection (dpi) and undetectable in lung lavage fluid at 5 dpi. The Ad5-HA vaccine induced a mucosal IgA response towards homologous CA09 virus and primed an antigen-specific T-cell response against both challenge viruses. Hemagglutination inhibiting titers were measurable in sera of WIV but not Ad5-HA vaccinated pigs. The WIV vaccinated pigs displayed vaccine associated enhanced respiratory disease (VAERD) following heterologous challenge (MN) characterized by enhanced macroscopic lung lesions and elevated non-neutralizing antibody titers to heterologous virus (MN) in nasal wash, BALF and sera. This study shows for the first time that a single intranasal vaccination with an Ad5 construct encoding the HA of influenza can provide complete protection to homologous challenge and partial protection to heterologous challenge, as opposed to VAERD, which can occur with adjuvanted WIV vaccine.

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Cytotoxic T cell responses in the spleen of infectious bursal disease virus infected chickens.
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Infectious bursal disease (IBD) is highly contagious disease of chickens which lead to immunosuppression of infected chickens. The IBD virus (IBDV) mainly infects IgM^+ B-cells. T cells are considered refractory to infection with IBDV but are known to promote virus clearance. However, the mechanisms of T cell mediated viral clearance are not well understood. In this study, we evaluated the cytotoxic T cell responses involving Fas-Fas ligand (FasL) and perforin-granzyme pathways in spleen of IBDV-infected chickens. Infection of chickens with IBDV was accompanied by the influx of $CD8^+$ T cells in the spleen. There was an upregulation in the gene expression of cytolytic molecules: Fas-Fas ligand (FasL), perforin (PFN) and granzyme-A (Gzm-A). Caspase-3 and PFN producing $CD8^+$ T cells were also detected in the spleen of IBDV-infected chickens by immunohistochemistry. The Th1 cytokine, IFN-γ was also detected, suggesting that the infiltrating $CD8^+$ T cells were activated. These data provide new insights in to the pathogenesis of IBD and suggest that the cytotoxic T cells may be involved in the clearance of virus-infected cells from IBDV-infected chickens.

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Antiviral regulation underlying the activation status of porcine monocytic innate immune cells.
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Activation statuses of monocytic cells including monocytes, macrophages (AMs) and dendritic cells (DCs) are critical for antiviral immunity in regulation of inflammation, tissue repair, antimicrobial activity and T-cell responses. Unfortunately, there are no studies on the activation status of porcine monocytic cells or how cell activation status modulates antiviral immunity. This is a significant omission because many porcine viruses are monocytotropic including, porcine reproductive and respiratory syndrome virus (PRRSV). Using cytokine multiplex assays, we showed that porcine monocytic cells could be skewed to various activation states, similar to M1 and M2 described in mice and humans. The purpose of this study was to determine the permissiveness and cytokine profiles following PRRSV infection in monocytic cells at different activation statuses. First, cells at different activation status showed dramatic differences in permissiveness to PRRSV infection. For example, M2 cells were more permissive to PRRSV infection. Second, cells at different activation statuses responded differently to PRRSV infection/exposure in cytokine secretion, which may result in different immune regulation. In summary, we have determined the activation status of porcine monocytic cells and the relationship of these statuses with PRRSV infection. These findings suggest that integration of activation status with antiviral responses in porcine monocytic innate immune cells may allow functional modulation, thus facilitating the development a prototypic cellular adjuvant/vaccines for potentiating antiviral immunity.

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Differentiation and immunoregulatory characteristics of porcine lung mesenchymal stem cells.
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Mesenchymal stem cells (MSCs) have self-renewing and differentiation potential and show potent immunosuppressive capacities *in vitro*. MSCs are normally isolated from bone marrow. However, exsistence of MSCs have been shown in other tissues, including the lung. In the present study, we isolated and characterized MSCs from porcine lung. Lungs were harvested from 4-6 week old germ-free pigs. Lung-MSCs grew as foci with a fibroblast like morphology, expressed stem cell markers, Oct4 and SSEA-1 and mesenchymal markers; CD29, CD44 and CD90. The cells were capable of osteogenic and adipogenic differentiation. We also examined the immunomodulatory properties of porcine lung MSCs. Like human lung-derived MSCs, porcine lung-MSCs profoundly suppressed the proliferative capacity of T cells in response to a mitogenic stimulus. Co-culture of MSCs with dendritic cells and T cells caused these cells to produce decreased levels of tumor necrosis factor alpha and interferon gamma respectively. Lung-MSCs produced elevated prostaglandin E2 (PGE2) in co-cultures, and inhibitors of PGE2 substantially abrogated MSC-mediated immune modulation. The results of this study demonstrate that MSCs derived from lung of pigs have *in vitro* immunosuppressive properties and differentiation potential. Future studies will focus on the role of lung-MSCs in tissue regeneration/remodeling in lung-injury models.

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Sandwich -ELISA for diagnostics of African Swine Fever.
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Purpose: African Swine Fever (ASF) is an infectious viral disease that causes high economic losses. The virus is spread by pigs, wild boars, ticks, through meat products. There is no treatment and no vaccine for ASF. Rapid changing of the virus into less virulent form leads to an endemic situation in the area. By clinical signs, ASF is similar to Hog Cholera. Experience of eradication of ASF in Spain suggests the importance of serological monitoring of pigs.The goal of this research was to develop and use a sandwich- ELISA method to detect ASF antigen based on a set of new monoclonal antibodies directed to independent epitopes of major capsid protein of ASF - vp73.
Methods: A recombinant fragment of vp73 protein containing conformational epitope was obtained and characterized in gel staining, immunoblotting,

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ELISA. This protein was purified using Ni-TNA agarose and used as antigen to obtain monoclonal antibodies. Two out of 4 clones with maximum activity were selected for sandwich ELISA, both being specific to vp73 in indirect ELISA.
Results: The indirect ELISA based on selected monoclonal antibodies can detect as low as 5-10 ng/ml of recombinant vp73 with minimal background. To access diagnostic sensitivity and specificity of the method, we tested blood serum samples, 10% tissue suspension from experimentally infected animals, and reference samples from historical outbreaks. To compare the results, samples were previously analyzed according to "Manual of Diagnostic Tests and Vaccines for Terrestrial Animals (mammals, birds and bees)". Chapter 2.8.1. OIE, six edition, 2008. The diagnostic sensitivity was 100%, specificity was 90% at optimized "cut-off" value selection.
Conclusions: The sandwich ELISA based on monoclonal antibodies to recombinant vp73 could be used to detect ASF virus for diagnostics and monitoring purposes.

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Fc expressed on the surface of the PED virus enhanced immunogenecity.
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Purpose:In this study, we make Fc molecules expressed Vero cell using swine IgG Fc gene expression vector and porcine epidemic disease virus(PEDV) grown in genetically modified Vero cells and PED virus may acquire Fc on the viral envelop. We test the immunological enhancement of the Fc molecules acquired PED virus as an inactivated antigen.
Methods: Vero cells were transformed with Fc expression vector and transformed cells were selected in the Zeocine containing media. PED virus propagated on the transformed Vero cells. Fc molecules on the Vero cell and PED virus were tested by ELISA, Western blot analysis and immunofluorescent analysis. Immunological enhancement test of the Fc harboring PED virus(PED-Fc) was performed using the titration of the serum antibody of the mouse immunized with PED-Fc virus. Immune cell proliferation, cytokine secretion, Serum neutralization antibody titer were measured in the serum of the pig immunized with PED-Fc virus.
Results: Immunofluorescent analysis showed that transformed Vero cells successfully express swine IgG Fc molecules on the cell membrane. PED virus harboring Fc molecules on the virus envelop was identified by ELISA assay. PED-Fc virus showed more strong immune stimulations in the mouse immunization test. IgG antibody titer increase 2 times, virus neutralization titer increase 16 times compare with normal PED virus. At the T cell proliferation assay also show PED-Fc virus increase 3 times compare with normal PED virus. Immunization with PED-Fc virus showed higher IgG2a/IgGl ratio comparing with normal PED virus. In the swine model, when the animals are immunized with PED-Fc virus(10^6 $TCID_{50}$/ml), serum neutralization antibody titer was 64 through 128. This SN titer 4 times higher than that of the same does normal PED immunization pigs.
Conclusions: In this study, we found that PED-Fc virus elect more strong immune responses compare with normal PED virus. PED-Fc virus immunized mouse and pig show more strong humoral as well as cellular immune responses. In addition, the newly established vaccines strategy might be able to apply for other viruses, which contain host cell derived envelope, using Vero cells or other host cells expressing Fc molecules.

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Adaptationof a commercial PRRS serum antibody ELISA to oral fluid specimens.
A. Kittawornrat[1], J. Prickett[1], C. Wang[1], C. Olsen[1], C. Irwin[1], Y. Panyasing[1], A. Ballagi[2], A. Rice[2], J. Johnson[1], R. Main[1], C. Rademacher[3], M. Hoogland[3], J. Zimmerman[1];
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Objective: Oral fluid samples are increasingly used for the surveillance of PRRSV infection in commercial swine operations using PCR-based assays (Chittick et al., 2011; Kittawornrat et al., 2010). While PCR-based assays are useful for detecting the circulation of PRRSV, antibody-based assays are informative
regarding herd immunity and history of prior infection. The feasibility of detecting antibody in oral fluids has already been addressed, i.e., antibody-based assays using oral fluid specimens are already widely available in human diagnostic medicine for a variety of pathogens (Prickett et al., 2010). The purpose of the present study was to optimize a commercial PRRS ELISA (HerdChek® PRRS X3 ELISA) to the
oral fluid matrix.
Methods: ELISA parameters assessed in the optimization process included: sample volume, sample dilution, incubation time, secondary antibody isotype (IgM, IgA, $IgG_{H\&L}$, IgG_{Fc}), and secondary antibody dilution. To reduce oral fluid sample-to-sample response variation during this process, 11 oral fluids ("Reference Standards") were used in the optimization process to measure the effects of changes in parameters. Reference standards were collected from one commercial wean-to-finish barn (1,100 pigs) prior to the day of PRRS vaccination (Ingelvac® PRRS MLV) and on DPV 0, 10, 15, 20, 28, 35, 41, 49, 56, 75, and 91. (Reference standards available upon request.)
Results: The PRRS ELISA (HerdChek® PRRS X3 ELISA) was readily adapted to the detection of IgM, IgA, and IgG in oral fluid specimens. The protocol developed for detection of IgG is readily amenable to the routine performance of the assay in high-throughput diagnostic settings.
References: Chittick WA, Stensland WR, Prickett JR, et al. 2011. Comparison of RNA extraction and RT-PCR methods for the detection of PRRSV in porcine oral fluid specimens. J Vet Diagn Invest 23:248-253.
Kittawornrat A, Engle M, Johnson J, et al. 2010. PRRSV in serum and oral fluid samples from individual boars: Will oral fluid replace serum for PRRSV surveillance? Virus Res 154:170-176. Prickett JR, Zimmerman JJ. 2010. The development of oral fluid-based diagnostics and applications in veterinary medicine. Anim Health Res Rev 11:207-216.

RESPIRATORY DISEASES

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Diagnostic performance of a commercial PRRS serum antibody ELISA adapted to oral fluid specimens.

A. Kittawornrat[1], J. Prickett[1], C. Wang[1], C. Olsen[1], C. Irwin[1], Y. Panyasing[1], A. Ballagi[2], A. Rice[2], J. Johnson[1], R. Main[1], C. Rademacher[3], M. Hoogland[3], J. Lowe[4], J. Zimmerman[1];
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Objective: Swine oral fluid samples are of interest because of their ease of collection and documented use in surveillance of PRRSV and other pathogens (Kittawornrat et al., 2010; Prickett et al., 2011). Previous work showed that a commercial PRRS ELISA (HerdChek® PRRS X3 ELISA) could be adapted to detect anti-PRRSV IgG in oral fluid specimens (IgG ELISA). The objective of the current study was to evaluate the ability of the assay to detect anti-PRRSV IgG antibody in pen-based oral fluid field samples.
Methods: Positive samples were derived from a longitudinal field study in 10 wean-to-finish barns (Ramirez et al., 2011). At each site, oral fluid samples were collected from the same 6 pens at 2-week intervals (total of 10 sampling points per barn). Positive oral fluid samples were defined as all samples collected from a pen after the first PRRSV PCR positive oral fluid sample from that pen (n = 241). Negative oral fluid (n = 284) field samples were diagnostic samples submitted to the ISU VLD for PRRSV qRT-PCR testing from expected-negative herds.
Results: Of 284 expected-negative field samples, all were negative on the IgG ELISA (S/P <0.40). 217 of 241 expected positive samples were positive. The 24 negative results on expected positive samples were from pens that initially tested positive and became negative over time.
Conclusion PRRSV IgG antibodies can be effectively detected in pen-based oral fluids samples using the commercial PRRS ELISA (HerdChek® PRRS X3 ELISA).
References: Kittawornrat A, Engle M, Johnson J, et al. 2010. PRRSV in serum and oral fluid samples from individual boars: Will oral fluid replace serum for PRRSV surveillance? Virus Res 154:170-176. Prickett JR, Johnson J, Murtaugh MP, et al. 2011. Prolonged detection of PCV2 and anti-PCV2 antibody in oral fluids following experimental inoculation. Transbound Emerg Dis 58:121-127. Ramirez A, Wang C, Prickett JR, et al. 2011. Efficient surveillance of pig populations using oral fluids. Prev Vet Med (submitted)

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Diagnostic performance of a commercial PRRS serum antibody ELISA adapted to oral fluid specimens: longitudinal response in experimentally-inoculated populations.

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Objective: Previous work in our laboratory showed that a commercial PRRS ELISA (HerdChek® PRRS X3 ELISA) could be adapted to detect anti-PRRSV IgM, IgA, and IgG in oral fluid specimens. Further, the protocol for the IgG ELISA for oral fluid samples was readily amenable to the routine performance of the assay in high-throughput diagnostic laboratories. This suggested the possibility of a cost-effective method to routinely monitor commercial swine populations for maternal antibody, vaccination compliance, and herd immune parameters using oral fluid sampling. The purpose of the present study was to evaluate the ability of the PRRS oral fluid IgG ELISA to detect anti-PRRSV IgG antibody in pen-based oral fluid samples from experimentally inoculated pigs over time.
Methods: In seven trials, ~200 pigs per trials were intramuscularly (IM) and intranasal (IN) inoculated with PRRSV isolate NVSL 97-7895. Oral fluid samples were collected on 0, 5, 7, 9, 11, 14, 17, and 21 days post inoculation (DPI). All oral fluid samples were randomized and tested for anti-PRRSV antibodies using the commercial PRRS ELISA, but following a protocol modified for the detection of anti-PRRSV IgG in oral fluids.
Results: Anti-PRRSV IgG antibodies were detected as early as 7 DPI and all samples were positive (S/P $\geq$ 0.4) by DPI 9. All samples remained positive through the end of study at DPI 21.
Conclusion: These results indicated that the ontogeny of anti-PRRSV antibodies in oral fluid is rapid and amenable to timely detection of PRRSV infection in the field. Testing based on oral fluid specimens could provide an efficient, cost-effective approach to PRRSV monitoring in commercial herds and surveillance in elimination programs.

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Evaluation of herd exposure methods to produce PRRSv-negative pigs from infected breeding herds.

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Purpose: This study compared the effectiveness of administering PRRS modified live virus vaccines to that of wild-type resident virus when used as part of a herd closure/stabilization program.
Methods: Eligible farrow-to-wean breeding herds that became infected with PRRSv and adopted herd stabilization programs to eliminate PRRSv were enrolled in the study. Time to produce PRRSv-negative pigs at weaning (TTNP) was compared from herds that used modified live virus (PRRS Ingelvac ATP or MLV, Boehringer Ingelheim Vetmedica, Inc.) to herds that used the wild-type resident virus (Batista et al., 2002). Herds were defined as producing PRRSv-negative piglets when they reached 90 days of consecutive monthly PCR-negative results. Preliminary descriptive analysis indicate that PRRSv shedding in farms going through herd closure is intermittent, indicating that PRRSv-monitoring must be done systematically over time. A total of 33 herds have been enrolled in the study at this time.
Results: From 20 farms with ongoing PRRSv monitoring, 12, 2 and 2 farms had respectively 1, 2 and 3 month of PRRSv PCR-negative results followed by PCR-positive results. In the farms with 2 or 3 months of PCR-negative results followed by PCR-positive results(n=4),phylogenetic comparison of the PRRSv isolates suggested that no new virus was introduced in those farms. As of the writing of this abstract,4 farms achieved TTNP. In the MLV group the TTNP periods for two farms were of 12.3 and 33.5 weeks and for the wild-type virus group,27.1 and 29.9 weeks. Increasing the amount of data in the study's database will enable us to better characterize the effectiveness of herd stabilization methods allowing producers to make informed decisions about PRRSv control and elimination.
Conclusions: Preliminary data show important observations including (a) the intermittent pattern of PRRSv shedding enforcing the need of repeated PRRSv-monitoring in herds going through herd closure and (b) TTNP can vary substantially among herds.

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Evaluation of immune responses to porcine reproductive and respiratory syndrome virus in pigs at day two post-infection.

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Porcine reproductive and respiratory syndrome virus (PRRSV) causes a persistent, economically devastating disease in pigs of all ages. Irrespective of regular vaccinations, control of PRRS has become a difficult task to swine farmers, and remained as a challenge. To comprehensively elucidate both cellular and innate immune cytokine responses at very early stage of PRRSV infection, seven weeks old pigs in a commercial research setting were infected and analyzed. One pig in a pen of 25 was PRRSV infected and responses were assessed two days later. All the 25 infected and a few of the 25 contact neighbor pigs were viremic. A majority of viremic pigs had more than 50% reduction in NK cell mediated cytotoxicity. At day 2 post-infection, in plasma nearly one fold increase in innate IFN-alpha production was detected. An enhanced secretion of IL-4, IL-10, and IL-12 (but not IFN-gamma in a majority of infected pigs was also observed. In addition, a reduced frequency of myeloid cells, CD4-CD8+ and CD4+CD8+ T cells and upregulated frequency of Foxp3+ T-regulatory cells were detected in viremic pigs. Interestingly, all the viremic contact pigs also had comparable immune cell modulations. The replicating PRRSV present in both infected and incontact pigs was found to be responsible for rapid modulation in NK cell function and modulation in secretion of innate immune cytokines, resulting in rapid subversion of host innate immunity. As the study was performed in pigs maintained in natural commercial environmental settings, results of this study have practical implications in developing protective PRRSV vaccines. This project is supported by USDA-NIFA PRRS CAP2 award to MPM and RJG.

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Attenuation of a virulent North American PRRS virus isolate on CD163-expressing cell lines, and demonstration of efficacy against a heterologous challenge.

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The discovery of the CD163 PRRSV receptor in 2004 has permitted the development of PRRS-permissive cell lines from various species. Attenuation of virulent field viruses on these cell lines has the potential to yield new modified live vaccine viruses that differ from existing monkey-cell attenuated vaccines. In one such example, the virulent genotype 2 US strain P129 was isolated directly from serum on a recombinant CD163-expressing pig kidney cell line. Following adaptation/attenuation for 24 passages, this virus was evaluated in a small study in which it showed satisfactory safety and efficacy against a heterologous challenge in a young pig respiratory model. Beginning at passage 31 the virus was adapted to a more manufacturing-friendly CD163-expressing cell line, derived from the baby hamster kidney cell line BHK21. With additional rounds of passaging, viral titers increased. At passage 57 the vaccine virus was evaluated for efficacy in a dose-titration study. Five groups of 24 pigs were mock-vaccinated or vaccinated with various doses of the P129 vaccine virus (targeting 1.5, 2.5, 3.5, and 4.5 $\log_{10}$ $TCID_{50}$/2 mL dose) at approximately three weeks of age and challenged with virus NADC20 at approximately seven weeks of age. Following vaccination, all pigs in all vaccinated groups seroconverted by ELISA. After challenge, vaccinated pigs had minimal clinical signs, and the three groups receiving higher vaccine doses were significantly ($p \leq 0.0132$) heavier than the unvaccinated control group. Lung lesions were assessed at necropsy (10 days post-challenge), and were significantly ($p \leq 0.0233$) reduced in all vaccinated groups. This study illustrates the utility of recombinant CD163-expressing cell lines in the generation of modified live PRRS vaccines. The animal use protocol was approved by the site Animal Care and Use Committee prior to initiation of the study.

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Antiviral effect of various mutagens against PRRS Virus.

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Modified live vaccines (MLVs) are widely used to protect PRRS virus infection because inactivated vaccines do not provide sufficient protection against PRRSV. Because PRRSV mutates at a high rate in nature and MLV can revert to virulent wild-type virus, the safety of MLV has become a great concern. In the current study, the replication of VR2332, a prototype of North American PRRS virus, in MARC-145 cells was assessed in the presence of four different types of antiviral mutagens (Ribavirin, 5-Fluorouracil, 5-Azacytidine and Amiloride hydrochloride hydrate) which are known to increase mutation frequency of RNA viruses above the tolerable error threshold during viral replication and drive the viruses into extinction. To determine the effect of mutagens on PRRSV replication in MARC-145 cells, the cells were treated with each mutagen before or after virus inoculation (B or A method, respectively). For B method, MARC-145 prepared in 48 hrs was incubated with growth media containing each of four antiviral mutagens at the concentration of 0, 0.2, 0.4, 0.6, 0.8 and 1.0 mM for 4 hrs before virus inoculation. On the other hand, the cells were replenished with growth media containing each of the antiviral mutagens at the same 6 concentrations described above after virus inoculation for A method. Then supernatants were collected every 24 hrs up to 4 days and tested for virus titration. In addition, cytotoxicity of the mutagens in MARC-145 was measured by a cytotoxicity assay. Among the four different mutagens, Ribavirin and 5-Fluorouracil effectively suppressed the replication of PRRSV without causing high cytotoxicity, whereas 5-Azacytidine and Amiloride hydrochloride hydrate showed antiviral effect against PRRSV replication but were highly cytotoxic. As Ribavirin and Fluorouracil have significant antiviral effect against PRRSV without causing high cytotoxicity, these might be good candidates for therapeutic agents against PRRSV infection though their efficacy and safety need to be evaluated based on *in vivo* assessment.

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Lipid-anchored membrane association of the PRRS virus GP4 glycoprotein and co-localization with CD163 in lipid rafts.

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The porcine reproductive and respiratory syndrome virus (PRRSV) glycoprotein 4 (GP4) resembles a typical type I membrane protein in its structure but lacks a hydrophilic tail at the C-terminus suggesting that GP4 may be a glycosylphosphatidylinositol (GPI)-modified lipid-anchored membrane protein. Using the human decay-accelerating factor (DAF; CD55), a known GPI-anchored protein, chimeric constructs were made to substitute the GPI-anchor domain of DAF with the putative lipid-anchor domain of GP4, and their membrane association and sensitivity to lipase were determined in cells. The DAF-GP4 fusion protein was transported to the plasma membrane and was cleaved by phosphatidylinositol-specific phospholipase C (PI-PLC) digestion, indicating that the C-terminal domain of GP4 functions as a GPI anchor. Mutational analysis of residues flanking the presumptive GPI modification site and characterization of the mutant viruses generated from infectious cDNA clones show that the viability and growth characteristics of the mutant viruses correlated with the ability of the GP4 to associate with the membranes. The residues T158 (ω-2, where ω is the GPI attachment site at E160), P159 (ω-1), and M162 (ω+2) of GP4 were determined to be important for virus replication, with M162 of particular importance for virus infectivity. The complete

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removal of the peptide-anchor domain of GP4 resulted in the loss of virus infectivity. Remarkably, GP4 was found to co-localize with CD163 in the lipid rafts on the plasma membrane. Since CD163 has been reported as a cellular receptor for PRRSV, our data implicates an important role of lipid rafts during entry of the virus.

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Adaptation of a commercial blocking ELISA to the detection of antibodies against influenza A virus nucleoprotein (NP) in porcine oral fluid specimens.
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Purpose: In commercial swine populations, influenza is an important component of the porcine respiratory disease complex (PRDC) and a pathogen with a major economic impact on swine production. Previously, a commercial blocking ELISA (MultiS-Screen® Antibody Test Kit, IDEXX Laboratories, Inc., Westbrook, Maine) designed to detect anti-influenza A nucleoprotein (NP) antibodies in avian serum was shown to effectively detect NP antibodies in swine serum (Ciacci-Zanella et al., 2010). The purpose of present study was to determine whether the "NP ELISA" could also be adapted to the detection of anti-NP antibodies in swine oral fluid samples.
Methods: The procedure for performing the oral fluid NP ELISA was modified from the manufacturer's instructions for testing serum by changing sample dilution, sample volume, incubation time, and incubation temperature. Using the modified procedure, the ability of the NP ELISA to detect influenza antibodies in pen-based oral fluid samples was evaluated using oral fluid samples (n = 182) from pigs inoculated with either influenza A virus subtype H1N1 or H3N2 under experimental conditions and followed for 42 days post inoculation (DPI).
Results: Anti-NP antibodies in oral fluid were detected from DPI 7 through DPI 42 in all inoculated groups. More specifically, the mean sample-to-negative (S/N) ratio of influenza-inoculated pigs was significantly different ($p < 0.0001$) from unchallenged controls (either unvaccinated or vaccinated-unchallenged groups). Oral fluid vs. serum S/N ratios from the same pen showed a correlation of 0.796 (Pearson correlation coefficient, $p < 0.0001$).
Conclusions: The results showed that the commercial NP ELISA has the potential to detect anti-NP antibodies in pen-based oral fluid samples. This approach could be used to monitor the circulation of influenza virus in commercial populations or time the use of vaccines in order to avoid maternal antibody interference.

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Emergence of novel reassortant H3N2 swine influenza viruses with the 2009 pandemic H1N1 genes in the United States.
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Purpose: Since the introduction of 2009 pandemic H1N1 virus (pH1N1) into swine, reassortant H1 swine influenza viruses (SIVs) with pH1N1 have been isolated from pigs in several Asian and European countries. Herein, we investigated whether reassortment occurred between pH1N1 with endemic SIVs in pigs in the United States.
Methods: Lung tissues and nasal swab samples were collected from diseased pigs in several commercial swine farms with outbreaks of respiratory disease in nursery pigs in the Midwestern USA from 2010 winter to 2011 spring. Influenza A virus was detected and isolated from samples by standard real-time RT-PCR and virus isolation. The isolated viruses were subtyped by hemagglutinin inhibition and gel-based RT-PCR assays with standard methods, and tested by the pH1N1 M-gene specific real-time RT-PCR. The isolated viruses were sequenced and analyzed by BLAST and phylogenetic tree to determine the source of the isolates.
Results: Total 20 SIVs were isolated from samples collected from diseased pigs. Six isolates were identified to be the H1N1 and 4 isolates were the H1N2 and 10 isolates were the H3N2 subtype. Seven H3N2 viruses out of 20 isolates were positive for the pH1N1 M-gene specific real-time RT-PCR and the full-length genome sequences of these 7 viruses were analyzed. Genome analysis showed that 6 H3N2 viruses contained NP, M and NS genes from pH1N1 and one isolate had PA, PB2, NP, M and NS from pH1N1, and the remaining genes are from endemic H3N2 triple reassortant SIVs. Phylogenetic analysis revealed that NP, M and NS genes of 7 novel H3N2 reassortant viruses grouped within the pH1N1 cluster. PB2 and PA genes of one H3N2 also clustered into the pH1N1 group. HA and NA genes of these 7 viruses belonged to triple reassortant H3N2 lineage. In addition, all the other internal genes from these 7 H3N2 viruses grouped within triple reassortant SIVs cluster.
Conclusions: Seven H3N2 isolates were novel reassortant viruses between pH1N1 and North American endemic SIVs. The emergence of novel reassortant H3N2 SIVs in US swine is further evidence of reassortment between pH1N1 and endemic SIVs and warrants continuous surveillance.

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Evaluation of cross-protection of FluSure XP® against a heterologous gamma cluster H1N1 swine influenza virus challenge.
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Purpose: Data from field surveillance indicate variability in the hemagglutinin (HA) gene sequence among contemporary gamma cluster H1 swine influenza viruses (SIV). This study evaluated efficacy in pigs vaccinated with the updated tetravalent formulation of a Swine Influenza Vaccine, H1N1, H1N2, & H3N2, Killed Virus (FluSure XP) and challenged with a contemporary gamma cluster H1N1 virus that is 91.8% similar to the gamma H1N1 vaccine virus in FluSure XP, based on HA sequence similarity.
Method: SIV-negative pigs (3 - 4 week old), allocated into two treatments (T01 placebo or T02 vaccinate) were vaccinated on Days 0 and 14. Pigs were challenged on Day 29 with a gamma cluster H1N1 field virus (A/Swine/Ohio/002973/2010) and necropsied on Day 34. Variables analyzed included macroscopic lung lesions at necropsy, hemagglutination inhibition (HI) antibody titers, virus isolation from bronchial alveolar lavage (BAL) fluids and clinical observations. The animal phase of this study was conducted according to the guidelines of Pfizer Animal Health's Institutional Animal Care and Use Committee.
Result: Compared to T01 placebo controls the T02 vaccinates had significantly lower percent lung lesions at necropsy ($P \leq 0.0001$). Virus was isolated from 61.1% of BAL fluid samples from the T01 pigs, but from none of the pigs in T02, with a significant reduction in the percent of positive pigs ($P \leq 0.0001$) and in the least squares mean viral titers ($P=0.0002$) for the vaccinated pigs. Compared to the placebo controls, vaccinated pigs responded with significantly higher HI antibody titers to all vaccine viruses ($P \leq 0.05$), but with the exception of one T02 pig, the pre-challenge HI titers to the Ohio challenge virus were <40. At necropsy on Day 34, the HI titers to the Ohio challenge virus were significantly higher ($P \leq 0.05$) in vaccinates (GMT=74.1) compared to placebo controls (GMT=6.8), suggesting that the pigs were primed to the heterologous challenge virus by the vaccine.
Conclusion: Under the conditions of this study, FluSure XP helped to protect from lung lesions, virus isolation from BAL fluids and clinical signs of respiratory disease in pigs experimentally challenged with a contemporary gamma cluster H1N1 SIV

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Modified live virus vaccine induces a distinct immune response profile compared to inactivated influenza A virus vaccines in swine.

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[1]National Animal Disease Center, Ames, IA, USA, [2]University of Maryland, College Park, MD, USA.

Purpose: The need for cross-protective influenza A virus vaccines in swine is necessary as the viruses circulating in swine become more genetically and antigenically diverse. This study compared the humoral and cell-mediated immune response of modified live virus (MLV) vaccine, inactivated adjuvanted virus (INV) vaccine, and priming with wild-type virus (WTV) infection.
Methods: Thirty-two influenza naïve pigs were allocated to four groups consisting of a MLV, INV, WTV and sham-vaccinated (SHV) group. The MLV and WTV groups were given two intranasal doses of 10^6 $TCID_{50}$/ml of an attenuated or wild-type A/Sw/MN/02011/08 δ-cluster virus (MN08), respectively. The INV group received 128 HA units in two intramuscular doses of UV-inactivated MN08 with adjuvant. Immune responses were evaluated with homologous MN08 and heterologous pandemic A/California/04/2009 H1N1 (CA09).
Results: Elevated numbers of interferon-γ secreting cells to MN08 and CA09 were detected in the MLV and WTV groups compared to the INV and SHV groups. Hemagglutination inhibition (HI) and serum neutralizing (SN) anti-MN08 vaccine strain antibody responses detected in the MLV and WTV groups after first vaccination were not detected in the INV group. All vaccinated groups demonstrated similar HI and SN antibodies after the boost vaccination. Virus neutralizing (VN) anti-MN08 antibodies were only detected in the bronchoalveolar lavage fluid (BALF) of the MLV and WTV pigs post boost vaccination. Nasal wash (NW) anti-MN08 IgG and IgA antibodies in the upper respiratory tract were highest in the MLV pigs after two doses compared to the WTV and INV groups. No cross-reactive anti-CA09 heterologous challenge strain HI, SN, BALF VN or NW IgG or IgA antibodies were detected in any vaccinated group. The MLV, WTV and INV groups demonstrated similar anti-MN08 IgG and IgA BALF antibodies. However, MLV and WTV vaccinated pigs demonstrated a more robust anti-CA09 IgG and IgA BALF antibody response compared to the INV group.
Conclusions: Collectively these data suggest the MLV vaccine induced a broader, locally acting adaptive (humoral and cellular) immune response against homologous vaccine and heterologous challenge strains.

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Evaluation of a commercial blocking ELISA kit for detection of influenza A nucleoprotein antibodies in canine sera.

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Purpose: Recent reports indicate dogs are susceptible to infection by influenza A viruses originating from horses (H3N8), avian species (H3N2, H5N1), and humans (pandemic H1N1). Infections are serologically diagnosed using subtype-specific hemagglutination inhibition (HI) and serum microneutralization (MN) assays, which are technically challenging and unsuitable for large-scale surveillance of infection by any virus subtype. Surveillance in other species depends on detection of antibodies to the highly conserved influenza A nucleoprotein (NP); however, there is no such antibody assay approved for canine sera in the US. The objective of this study was to evaluate the diagnostic accuracy of an IDEXX blocking ELISA licensed for avian species in detecting influenza A NP antibodies in canine sera.
Methods: Sera from uninfected dogs (n=204) and dogs naturally infected with H3N8 canine influenza virus (n=150) were tested according to manufacturer instructions in the IDEXX FlockChek blocking ELISA. The sample/negative control (S/N) absorbance ratios were calculated. A receiver operating characteristic (ROC) curve analysis was conducted, using HI titers as the gold standard, to determine the S/N cutoff ratio based on the optimum diagnostic sensitivity and specificity. Coefficients of variation for intra-assay and inter-assay variability were also calculated.
Results: The S/N absorbance ratios ranged from 0.12 to 0.67 for infected dogs and 0.53 to 1.40 for uninfected dogs. Based on ROC curve analysis, the optimum diagnostic sensitivity (99.3%) and specificity (99.0%) was obtained at a S/N cutoff ratio of 0.647. At this cutoff, the overall diagnostic accuracy was 99.2%. Coefficients of variation for intra-assay (4.7%) and inter-assay (6.1%) variability demonstrated good repeatability.
Conclusion: The excellent diagnostic accuracy of this commercial blocking ELISA makes it a suitable tool for large-scale surveillance of influenza A virus exposure in dogs.

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Pneumonia cases associated with *Mycoplasma hyopneumoniae*: a retrospective evaluation of diagnostic cases from 2003 to 2010.

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

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Increased prevalence of torque teno viruses in porcine respiratory disease complex affected pigs.

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The role of swine Torque Teno viruses (TTSuVs) as co-factors in disease syndromes involving porcine circovirus strain 2 (PCV2) and porcine reproductive and respiratory disease syndrome virus (PRRSV) has been a debatable subject. In this study, the prevalence of TTSuVs in Iowa, the leading pork producing state in the U.S was estimated by a duplex PCR which was capable of simultaneously detecting both TTSuV1 and TTSuV2. Based on an analysis of 300 random samples representing six major geographical regions of the state, the overall prevalence rate for TTSuv1&2 were 47.34% and 24.67% respectively while the combined prevalence rate was 52.33%. The epidemiological association of TTSuV1&2 with the common etiological agents of the porcine

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respiratory disease complex (PRDC) namely porcine PRRSV, PCV2, *Mycoplasma hyopneumoniae* and swine influenza virus (SIV) was estimated in lung tissue from 45 pigs showing clinical signs of PRDC. Notably, 86.67% of the PRDC-suspect samples were positive for TTSuV1 when compared to the baseline population prevalence rate of 47.34%. However, the prevalence TTSuV2 (26.67%) was not significantly different. TTSuV1 was detected in 80.00%, 81.81%, 75.00% and 77.78% of the PRRSV, SIV, *M. hyopneumoniea* and PCV2 positive PRDC-suspect samples respectively. Therefore our results indicate that TTSuV1 is strongly associated with clinical PRDC. They support the hypothesis that TTSuVs might function as co-factors in PRDC. Further studies to define their possible role in the pathogenesis of swine respiratory diseases are warranted.

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The development and validation of two non-invasive diagnostic screening assays for the detection of tuberculosis infection in non-human primates.
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Purpose: The introduction of novel infectious diseases has become as a major threat to endangered primate populations. This is particularly true for habituated great ape populations, which have been conditioned for close encounters with human observers, and there is much evidence that exposure of primates to human pathogens, particularly those of respiratory origin, readily occurs. In an effort to sustain the health of habituated great ape populations, continued health monitoring of these populations is recommended. Unfortunately, such health monitoring for some diseases is hampered by a paucity of sensitive, non-invasive diagnostic assays. Tuberculosis, a disease of high prevalence among humans in many African regions, is an example and poses a significant health risk for habituated great ape populations. The goal of this project was to develop and validate fecal and urine enzyme-linked immunosorbant assays (ELISAs) for the detection of host and pathogen-derived biomarkers of *Mycobacterium tuberculosis* (M.tb) infection in non-human primates.
Methods: An ELISA was developed for the detection of a M.tb biomarker that is shed in the urine, lipoarabinomannan. This is an outer cell wall lipoglycan, specific for pathogenic mycobacteria of the M.tb complex (MTC). A second set of ELISAs were also developed to detect in feces, host antibodies to the highly antigenic proteins ESAT-6, Cfp10, and Ag85, all of which are specific to members of the MTC. ELISAs were developed using primate urine and fecal samples spiked with the target biomarkers, and validation of the assays was carried out with the testing of known M.tb positive and negative macaques (*Macaca* spp.). Receiver operating characteristic analyses were used to assess the diagnostic accuracy of the ELISAs.

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

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Genome-wide analysis of gene expression profile change in response to BRSV and *H. somni* in bovine respiratory epithelial cells.
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Bovine respiratory syncytial virus (BRSV) and *Histophilus (H.) somni* are synergistic pathogens in bovine respiratory disease (BRD). The mechanism(s) causing the synergy are unclear. To evaluate the interaction of these pathogens with bovine respiratory epithelium we evaluated the bovine genome-wide gene expression profile change and the protein production of selected molecules in response to BRSV and *H somni* in bovine alveolar type 2 (BAT2) cells. BAT2 cells were infected with BRSV or without BRSV for 60 hrs and then co-incubated with or without *H. somni* culture supernatants for 4 hrs. RNA was harvested for RNA amplification and labeling. Affymetrix Bovine Genome Arrays were used to profile gene expression patterns. Microarray data were analyzed using Web-based software. BAT2 cell culture supernatants were collected for measurement of selected protein production. Data were generated from three replicates. Differential gene expression was presented as fold-change of control (± 1.5, $p \leq 0.05$).
After BRSV infection 188 of 24,016 transcripts exhibited significant changes in gene expression, mainly genes involved in innate and pro-inflammatory responses. Gene expression after exposure to *H. somni* supernatant included 2276 genes (1176 genes were up- and 1100 were down-regulated). In addition to the pathways induced by BRSV, a large number of genes are involved in cell division and growth, apoptosis, and tissue repair and remodeling. Gene expression after dual treatment showed that 1091 genes were up- and 1279 genes down-regulated; some were regulated synergistically by BRSV and *H. somni*. These included IL-6, IL-8, MMP1 and 3, and prostaglandin synthase. Protein expression levels confirmed that those genes are up-regulated synergistically by BRSV and *H. somni* not only at the transcription level but also at the protein level.
In conclusion: a larger number of genes responded to *H. somni* than to BRSV. Interestingly, some genes involved in inflammation, wound repair and tissue remodeling were synergistically up-regulated by dual challenge with BRSV and *H. somni*. This new finding may suggest a new strategy for the development of the treatment and preventive measures for the BRD.

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BRSV and *H. somni* synergy in bridging the alveolar barrier.

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Histophilus somni and BRSV are important bovine respiratory pathogens that synergistically enhance severity of pneumonia in dually infected cattle. *H. somni* also causes septicemia with subsequent thrombotic meningoencephalitis, myocarditis, and arthritis. Septicemia likely results from *H. somni* crossing the alveolar barrier, composed of alveolar epithelial cells and the underlying basement membrane. We previously found that the *H. somni* virulence factor, IbpA, induced rounding and retraction of the alveolar epithelium, allowing paracellular migration of the bacteria through a monolayer of bovine alveolar type II (BAT2) cells. To investigate BRSV/*H. somni* synergy in bridging this barrier, we infected BAT2 cells with BRSV for 60 hours prior to infection with 10 MOI *H. somni*. We found a significant increase in the number of retracted and rounded cells after the dual infection as compared to treatment with *H. somni* alone. Transwell assays also demonstrated a significantly increased transmigration of *H. somni* across BAT2 monolayers infected with BRSV as compared to non-BRSV-infected BAT2 monolayers. After crossing the alveolar epithelial barrier, the bacteria pass through the basement membrane to gain access to the underlying microvasculature. Since BAT2 cell matrix metalloproteinases (MMPs) 1 and 3 are up-regulated in after treatment with *H. somni* IbpA- enriched concentrated culture supernatant (CCS) and synergistically further up-regulated in BRSV/*H. somni* CCS treated cells, we hypothesized that the increased production of MMPs by BAT2 cells would increase digestion of the basement membrane and subsequent invasion of the capillaries. Gelatin zymography demonstrated increased MMP activity in BAT2 supernatants after treatment with CCS as compared to treatment with media alone. Taken together, we propose that *H. somni* can migrate from the alveolus to the microvasculature by IbpA-induced retraction of BAT2 cells associated with increased epithelial production of MMPs to degrade the underlying basement membrane. This mechanism of *H. somni* invasion is synergistically enhanced by BRSV.

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Cytokine and chemokine responses of equine pulmonary alveolar macrophages are altered in a dose-dependent manner to *Rhodococcus equi* infection.

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Purpose: The objective of this study was to examine the effect of infectious dose on cytokine and chemokine gene transcription in Rhodococcus equi infected pulmonary alveolar macrophages from adults and foals.
Methods: Alveolar macrophages were obtained by bronchoalevolar lavage from 7 healthy mares and their 5-week-old foals. Primary macrophage cultures were infected with *R. equi* 33701+ or 33701- at a low dose (MOI of 1) or a high dose (MOI of 100). Uninfected cells were cultured as a media control. Cells were harvested at 4 and 24 hours post- infection. Total RNA was extracted using the RNAeasy kit (Qiagen, Valencia, CA) and cDNA synthesis was performed using the QuantiTect Reverse Transcription Kit (Qiagen, Valencia, CA). RT-PCR assays were performed on the Applied Biosystems 7300 Real Time PCR System (Foster City, CA). Relative gene transcript levels for IL-6, IL-12p40, TNFα and CXCL10 and GAPDH were calculated using the ΔΔCt method. Data was analyzed using ANOVA.
Results: Cellular infections at the higher dose resulted in significantly higher expression of IL-6, IL-12p40 and TNFα mRNA transcripts compared to the low dose. The dose-dependent effect was reversed for CXCL10 with significantly lower expression at the higher MOI when cells were infected with the virulent strain.
Conclusion: Significant down-regulation of CXCL10 mRNA transcripts associated with a higher dose is interesting as this chemokine plays a role in development of protective Th1 responses and may represent a novel mechanism by which *R. equi* modulates immune responses and therefore disease.

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Infections caused by *Rhodococcus equi* in foals: immunologic and therapeutic considerations.

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Pneumonia is the leading cause of morbidity and mortality in foals in the United States. *Rhodococcus equi*, a facultative intracellular pathogen, is the most devastating cause of pneumonia in foals and has a major financial impact on the equine industry. In contrast to foals, adult horses are resistant to infections caused by *R. equi*. Studies in mice have shown that a T helper (Th) 1 response, characterized by synthesis of IFN-gamma is essential for protection against *R. equi*. The documented Th2 bias in immune responses of murine and human neonates, along with the fact that young foals are deficient in their ability to produce IFN-gamma in response to mitogens, have led to the widespread belief that foals are born with little or no capacity to mount adequate Th1 responses. However, recent findings demonstrate that most foals have the ability to mount protective immune responses to *R. equi*. The basis for the peculiar susceptibility of foals to infection with *R. equi* is likely complex and multifactorial.
Control of *R. equi* infections on many farms where the disease is endemic currently relies on early detection of disease using thoracic ultrasonography and initiation of treatment with a combination of a macrolide and rifampin prior to development of clinical signs. However, mass antimicrobial treatment of subclinically affected foals is not without potential risks, including adverse effects and selection for antimicrobial resistance. We have recently shown that emergence of macrolide and rifampin resistance in *R. equi* is increasing and that foals infected with resistant isolates are less likely to survive. Recent evidence indicates that resistant isolates are widespread on farm where mass antimicrobial therapy has been used extensively. We have recently shown that many foals with small pulmonary abscesses recover without antimicrobial therapy and that antimicrobial treatment of subclinical lesions does not significantly hasten recovery, compared to administration of a placebo. As a result, mass antimicrobial therapy of all foals with small subclinical pulmonary abscesses, as currently practiced, appears unnecessary and may contribute to widespread antimicrobial resistance.

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Development of a sheep model for studying pathogen vector interactions of *Anaplasma phagocytophilum* and *Ixodes scapularis*.

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Purpose: *Anaplasma phagocytophilum* has been recognized as an emerging tick-borne pathogen of humans in the U.S. and Europe. While the pathogen is known to be transmitted by *Ixodes sp.*, the tick developmental cycle of *A. phagocytophilum* and the pathogen/vector interactions have not been fully described. Herein, we report development of a sheep/tick model of *A. phagocytophilum*.
Methods: The NY18 isolate of *A. phagocytophilum* was propagated in cultured HL60 cells and then used to infect sheep by intravenous inoculation. Infection of sheep was determined by real time RT-PCR and an *Anaplasma* competitive ELISA, after which they were infested with *Ixodes scapularis* ticks.
Results:Clinical symptoms were not apparent throughout the infection except for slight elevation of body temperature observed beginning at 8-14 days p.i. and lasting 4 to 12 days. Limited hematologic abnormalities were identified including mild lymphopenia and neutropenia. Blood film evaluation revealed prominent large granular lymphocytes, occasional plasma cells, and rare macrophages. While typical *A. phagocytophilum* morulae were not seen in neutrophils in stained blood films, rare cytoplasmic granulation was observed which may have represented small morulae. Mild serum biochemical abnormalities were noted, consisting of hypocholesterolemia, hypoalbuminemia, decreased CPK activity, and increased activity of ALP and GGT. Upon necropsy, gross lesions were mild and restricted to the lymphoid system. Mild splenomegaly and lymphadenomegaly with microscopic evidence of lymphoid hyperplasia was observed in each sheep. Minimal and patchy neutrophilic pneumonitis was microscopically apparent in two of the sheep. One sheep had moderate numbers of eosinophils in lymph node sinusoids and in perisinusoidal regions of the adrenal medullas. Twenty female *I. scapularis* that were allowed to feed and acquire infection on each of the three sheep developed *A. phagocytophilum* infections as determined by real time RT-PCR of gut (85 to 87%) and salivary gland (67% to 100%) tissues.
Conclusions: Sheep therefore serve as a good host for infection of ticks with *A. phagocytophilum* in order to study the tick/pathogen interface.

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Potentially protective dual oxidase enzymes (Duox1 and Duox2) in T.foetus infected bovine endometrial cells *in vitro*.

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Purpose: Vaginal and uterine mucosa is the portal for sexually transmitted diseases (STD) in animals and man which can lead to major economic costs. Understanding innate immunity at mucosal surfaces is essential to enhancing protection and controlling STD. The recent discovery of the dual oxidases (Duox1 and Duox2) capable of H2O2 associated killing of microbes in different mucosal membranes suggests that these enzymes may be important in the innate defenses of the reproductive tract. Previously we identified Duox1 and 2 in the reproductive tract of mice and cows (in vivo) as well as in the bovine endometrial cell lines (in vitro). The aim of the current study is to examine the production of Duox1 and 2 in vitro during acute T. foetus infection of cultured bovine endometrial cells.
Methods: Endometrial cell lines were infected with T.foetus for 6 hrs and the production of Duox1 and 2 in the cells was determined using immunohistochemistry and rtPCR.
Results: The mean critical threshold values (delta Ct) between the infected and the uninfected were compared indicating no significant change in Duox 1 and 2 levels.
Conclusion: This suggests that if these molecules play a role in innate immunity, it may be later than 6 hrs post-infection.

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Understanding the basis of strain-restricted immunity to Theileria parva.

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Purpose: Thelieria parva (T. parva) is the causative agent of East Coast Fever (ECF) and Corridor disease (CD), both being diseases of high economic importance in Africa. T. parva is an intracellular protozoal parasite which is transmitted via the three-host tick vector, Rhipicephalus appendiculatus (R. a.) from wild buffalo to cattle (CD) and between cattle (ECF). Current evidence suggests that though T. parva is genotypically diverse, buffalo-derived parasites are more diverse than those maintained in cattle. Further defining T. parva diversity is a significant aspect in developing a more complete understanding of the disease, and has the potential to contribute to improvements on the current vaccine. The infection and treatment method (ITM) of vaccination involves the inoculation of susceptible cattle with a cocktail of infected ticks containing three T. parva isolates, and simultaneous administration of long-acting Oxytetracycline. Cattle that recover from clinical infection with T. parva exhibit a strong but strain-specific immunity, which is believed to be mediated predominantly by cytotoxic T cells (CTL). Ten antigens have been identified, which are the targets of CTLs in immune cattle. The main experimental objective of this study was to determine the antigenic diversity of T. parva isolated from buffalo.
Methods: The sequences of the genes encoding the ten T. parva antigens (Tp1 - Tp10) were analysed in parasitized cell lines isolated from 32 naturally infected buffalo. Additionally, the sequences of known CTL epitopes within 8 of the antigens (Tp3 and Tp6 epitopes not defined) are also being analyzed.
Results: The results, together with previous published data, has defined polymorphisms within Tp1 andTp2 epitope regions. Results to date indicate no variation within the Tp4, Tp5 or Tp8 epitope regions amongst buffalo tested.
Conclusions: Data at abstract submission currently suggest that the CTL antigens from buffalo-derived parasites vary considerably in the degree of polymorphism they exhibit. The non-polymorphic epitope regions have the potential to be used in a broadly protective subunit vaccine based on the induction of a T. parva specific CTL response

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Prevalence of tick-borne anaplasmal pathogens among naturally infected client-owned dogs in Missouri.

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Several species in the rickettsial family Anaplasmataceae are causative agents of tick-borne zoonoses endemic to Missouri. Although these agents are also infectious to domestic dogs, the importance of natural transmission of these pathogens among domestic hosts in Missouri is poorly understood. The objective of this study was to investigate the potential role of the canine population in the epidemiology and monitoring of tick-borne anaplasmal zoonoses. Template was collected from canine blood samples submitted to the University of Missouri Veterinary Medical Diagnostic Laboratory Clinical Pathology Section between June 1, 2010 through May 30, 2011, divided according to six geographic regions of the state, and a minimum of 130 samples were randomized according to three four-month seasons. These 421 samples were tested with a 16S rDNA-based real-time PCR assay to detect tick-borne

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Anaplasmataceae among these client-owned dogs across Missouri. PCR-positive samples were confirmed with agarose gel electrophoresis, and amplicon sequence analysis was used to identify confirmed samples to the species level. The overall prevalence of tick-borne Anaplasmataceae among dogs across Missouri was over 10%. The importance of these results will be discussed in context of distribution, vector biology, serologic surveys and human infections with these different pathogens in Missouri.

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Epidemiology of epizootic hemorrhagic disease in white-tailed deer in Texas.
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Purpose: Epizootic Hemorrhagic Disease Virus (EHDV) is a vector-borne RNA-virus causing debilitation and death in deer. The distribution of EHDV serotypes in the United States has changed with the recent emergence of EHDV-6 in several states including Texas. The objective was to determine the effect of host factors (age and sex) and season on the risk of infection with EHDV and to investigate the spatio temporal distribution of EHDV and its serotypes in white-tailed deer in Texas.
Methods: Data was obtained from the Texas Veterinary Medical Diagnostic Laboratory on EHDV tests performed on white-tailed deer in Texas between 1999 and 2011. Diagnosis of EHDV infection was carried out by RT-qPCR while serotype was determined by multiplex PCR based on the highly variable segment-2 (VP2). The association between EHDV infection and putative risk factors was investigated using logistic regression. Retrospective space-time analysis was conducted using the Bernoulli model of the scan statistics.
Results: There were 1,436 EHDV test results with an overall proportion of positives of 23%. Univariable logistic regression suggested that older age and male sex were associated with an increased risk of infection with EHDV. As expected the risk was higher during the warmer months. Age and sex remained significant risk factors after controlling for season and year. Genotyping was performed on 177 specimens isolated between 2009 and 2011. While serotype EHDV-6 has been detected in 14 counties, 40% (18/45) of all isolates originated in a single county in south Texas. The scan statistics identified 2 space-time EHDV clusters. The most likely cluster occurred in 2011 in south Texas while the secondary cluster was detected in 2010 in north-central Texas. Interestingly, 81% (25/31) of all EHDV-6 isolates recovered in 2011 were located within the primary cluster, while none of the EHDV-6 isolates from 2010 were part of the secondary cluster.
Conclusions: Knowledge of host-related risk factors for EHDV aids the identification of high-risk populations. It is highly plausible that the recent clusters of EHDV in Texas involving different serotypes are due to changing ecological or climatic conditions.

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Functional analysis of tick genes differentially expressed in response to Anaplasma phagocytophilum infection.
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Purpose: The overall goal of this research was to characterize molecular interactions necessary for the vector competency of the tick, *Ixodes scapularis*, for *Anaplasma phagocytophilum*, an emerging tick-borne pathogen in the U.S. Proteomics was used to identify proteins differentially expressed in *I. scapularis* ISE6 tick cells in response to *A. phagocytophilum* infection. Methods: Analysis of differential protein expression demonstrated that biological processes (BP) such as cell growth, protein and nucleic acid metabolism, and transport were affected during early and late infections. However, differences were observed between under- and over-expressed proteins in both early and late infections for cell growth and transport BP and between early and late infections for cell growth, energy and lipid metabolism, and cell communication BP. Proteins in cell growth and transport BP were selected for the characterization of mRNA levels during *A. phagocytophilum* infection in ISE6 tick cells. Genes that exhibited significant differences in mRNA levels were selected for gene knockdown by RNA interference (RNAi) in order to test the effect of gene silencing on pathogen infection levels. Results: Six out of sixteen genes, CG2, CG8, CG10, T1, T2 and T3 were found to be downregulated by proteomics and real-time RT-PCR in cultured tick cells in response to *A. phagocytophilum* infection. While the silencing of CG2, CG10, T1 and T3 had no affect of *A. phagocytophilum* infection levels in cultured tick cells, the silencing of CG8 resulted in lower infection and the silencing of T2 caused an increase in infection. Conclusions: These results suggested that CG8 might be required for pathogen infection while T2 may be involved in the protective response of the tick to limit pathogen infection. Overall, this research extends our understanding of the tick-host-pathogen interface.

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Plasmepsins: Dissecting the function of a parasite's protease degradome.
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The genomes of intracellular parasites such as the malaria parasite encode large numbers of proteases many of which are essential to completing the life cycle of the parasite, or if non-essential, provide a survival advantage. Our study of the plasmepsins, the aspartic proteases of *Plasmodium* spp., offers lessons in dissecting the degradome and provides a look at the diverse functions of proteases with a common mechanism of action. The subcellular location and timing of expression of some of these proteases have provided critical information about their substrates, and thus their roles in cellular function and/or host-parasite interactions. Genetic modification, recombinant protein expression, computer modeling, crystallography and small molecule inhibitors have been used as tools to evaluate these proteases as targets for antiparasitic drug development. Some of our plasmepsin-deficient parasite lines demonstrate growth defects *in vitro*, reduced virulence in the mammalian host, and an altered host immune reaction. Studies of the plasmepsins thus have provided insights into the challenge of identifying enzymatic targets suitable for antiparasitic drug development and have demonstrated the ability of a single gene knockout to attenuate a highly pathogenic parasite resulting in an altered course of infection.

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Improvement in diagnostic specificity of *anaplasma marginale* msp5 epitope-based celisa with new antigen construct.
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Bovine anaplasmosis is a vector-borne rickettsial disease caused by *Anaplasma maginale*, *Anaplasma centrale* and *Anaplasma phagocytophilum*. Almost all outbreaks of clinical disease are related to the infection with *Anaplasma marginale*. Once infected, cattle become life-long carriers. Several serological assays such as complement fixation (CF), card agglutination, ELISA, and indirect fluorescent antibody tests were used in the detection of carriers. The CF test is no longer considered a reliable test due to low sensitivity. Card agglutination and PCR are also less reliable tests with variable sensitivities (67% and 34% respectively, J. Vet. Diagn. Invest., 22:192-199, 2010) than MSP-5 epitope-based cELISA (99%). The diagnostic specificity of the cELISA was 89%

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in the same study.
This study was carried out to test the hypothesis that maltose binding protein (MBP) included in MSP-5 recombinant antigen construct causes false-positive results. Sera from dairy herd kept in vector-controlled barns in the State of Washington were analyzed in the cELISA with and without MBP absorption plates to determine if elimination of MBP-binding immunoglobulin was complete. The prevalence of MBP binders was 39.7% (143 of 360 sera) in tested herds. The majority (95.8%) of these MBP binders were rendered negative by the MBP absorption procedure included in the cELISA. Six MBP binders and three other sera were still positive in the cELISA after MBP absorption showing at least 97.5% of specificity for the cELISA using the antigen from MBP fusion protein-containing expression system. However, only 1 of the 143 MBP binders was positive in the cELISA using new antigen expressed without the MBP fusion protein. None of the other three sera positive in the cELISA was positive in cELISA with new antigen.
If this result is confirmed by further study, the cELISA with new antigen will improve the diagnostic specificity of the assay by eliminating false-positive reaction due to MBP-binding antibodies and other non-specific antibodies in bovine sera.

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A novel *Theileria equi* sporozoite challenge model for pathogenesis and immune control studies in immunocompetent and immunodeficient horses.
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Purpose: Theileria equi is a tick-borne apicomplexan parasite of horses worldwide. The recent reemergence of T. equi in the U.S. has led to the characterization of two transmission-competent native tick species and demonstrates that current control strategies are insufficient to protect the U.S. horse population from this parasite. Novel intervention strategies are necessary and require a detailed understanding of parasite pathogenesis and a reproducible disease model. The objectives of this study were to characterize infected equine peripheral blood mononuclear cells (PBMC) *in vitro* and develop a novel infection model.
Methods: To characterize mononuclear cell infection, T. equi sporozoites were co-cultured with fresh PBMC. Cell culture infection was monitored by Geimsa staining of cytospin preps and IFA.
The *in vivo* pathogenicity of isolated sporozoites was tested by IV inoculating immunocompetent horses and foals with severe combined immunodeficiency (SCID). Disease status was monitored clinically and parasitemia was documented by real time PCR.
Results: *In vitro*, T. equi infected PBMC underwent schizogony and survived for 35-40 days. Intravenous inoculation of immunocompetent horses and SCID foals with T. equi sporozoites caused clinical theileriosis in all animals.
Conclusions: Although sporozoite infection of equine PBMC was confirmed *in vitro*, mononuclear cell transformation and persistent proliferation, as seen with Theileria parva, was not observed. These findings are consistent with *in vivo* T. equi infection, which, unlike T. parva, does not include mononuclear cell proliferation in its pathogenesis. Intravenous injection T. equi sporozoites into immunocompetent horses and SCID foals was shown to cause a consistent course of infection and significant clinical disease. Arabian SCID foals lack T and B lymphocytes; therefore, successful infection of SCID foals with sporozoites indicates that this subpopulation of mononuclear cells is not required for transmission. These models will allow immune responses against the pre-erythrocytic stages of T. equi to be characterized *in vitro* and protective effects determined *in vivo* in a rigorously controlled manner.

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Light microscopic study of the developmental cycle of *Ixodes scapularis*.
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Purpose: *Ixodes scapularis* is a medically important tick in North America because it vectors human pathogens, most notably *Borrelia burgdorferi*, the etiologic agent of Lyme disease and *Anaplasma phagocytophilum*, which causes the emerging tick-borne disease, human granulocytic anaplasmosis. Because ticks are necessary for transmission of these pathogens, recent research has been directed toward understanding the molecular basis of the tick/pathogen interface. RNA interference has become an important tool for genetic manipulation in ticks in order to assess the impact of silencing individual or groups of genes on tick biology and vector competency. Because phenotypic changes in tick morphology following gene knockdown may be subjective and not easily recognized, a more precise characterization of the development of *I. scapularis* is important toward documenting these changes.
Methods: In this study, the 25 week time-line of the *I. scapularis* life cycle was documented and light microscopy with improved optics was used to capture the normal morphology of mating, oviposition, and of each tick stage (larva, nymph and adult). Egg hatching, egg development and molting to the nymphal and adult stages were also studied microscopically.
Results: The life cycle of *Ixodes scapularis* was documented with light microscopy. In the laboratory, one generation of ticks can be produced in 7.5 months. Rearing of *I. scapularis* requires more detailed handling that other species of ticks.
Conclusions: The detailed description of the normal developmental cycle and morphology are fundatmental toward understanding and documenting the impact of gene silencing.

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DIVA Vaccination for Avian Influenza Virus: Ready for Prime Time?

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Vaccination for both low pathogenic and highly pathogenic avian influenza is commonly used for countries that have been endemic for avian influenza. Stamping out policies are common for countries that are normally free of the disease. The stamping out policies of identifying infected and at risk flocks, then euthanizing all the birds to prevent disease spread, has been an effective control tool, but it comes at a high social and economic cost. Countries that vaccinate are considered to be endemic for the disease and typically lose their export markets. As a tool to promote trade, the concept of DIVA (Differentiate infected from vaccinated animals) has been considered for avian influenza. The goal for trade is to differentiate vaccinated, not infected from vaccinated, infected animals, because trading partners are unwilling to buy infected birds. DIVA strategies include 1) the use of sentinel animals in flocks (tracking the sentinel animals is difficult), 2) the heterologous neuraminidase DIVA strategy (the idea is to match the hemagglutinin subtype in the vaccine to the field strain, but with a different neuraminidase subtype that can be used a differential test, 3) the non-structural gene (NS1) approach (the NS1 gene as a non-structural gene is not found in the viral particle, but it is produced in large amounts in infected cells but the NS1 protein antibody response is not as robust as other influenza genes), 4) the Matrix protein 2 (it suffers from similar problems to NS1 protein), 5) a positive marker approach using tetanus toxin (this approach does not differentiate vaccinated and vaccinated, infected animals), and 6) the viral vector class of vaccines (all these vaccines express the hemagglutinin proteins, but not internal proteins, and commonly used diagnostic tests for the nucleoprotein can be used to differentiate infected from just vaccinated animals). All these approaches have advantages and disadvantages, and none are internationally recognized. The desire for a workable DIVA strategy may push one of these ideas from experimental to the practical.

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Pathogenicity and cytokine gene expression patterns associated with fowl adenovirus serotype 4 infection.

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Inclusion body hepatitis hydropericardium syndrome (HPS) is a recently emerged infectious disease of poultry associated with some strains of fowl adenovirus serotype 4 (FAdV-4). This transmissible syndrome is clinically distinguishable from other adenovirus-induced diseases by accumulation of a jelly like fluid in the pericardial sac and by the high mortality up to 75% in infected flocks. HPS was initially reported from Pakistan, however outbreaks also occurred in India, the Middle East, Japan, Mexico, Peru, and Chile.
In this work, a Canadian isolate of FAdV-4 was evaluated for pathogenicity, and the effects of FAdV-4 infection on the transcription of a number of avian cytokines were studied *in vivo*. The FAdV-4 was recovered from a broiler breeder flock with no clinical signs of the disease. The virus was plaque purified and subjected to a pathogenicity study by oral and intramuscular (im) routes of infection of specific pathogen free (SPF) chickens. The pathogenicity was evaluated by observation of clinical signs, gross and histological lesions. The pattern of virus dissemination in tissues, such as liver, bursa of Fabricius and cecal tonsils by assessing copy number by quantitative PCR was also investigated. The highest viral copy numbers irrespective of the inoculation route were detected in the cecal tonsils. The virus titers in the cloacal swabs collected over the entire study period were compared between the orally and im inoculated chickens and the difference in titers between the two groups was significant ($P<0.001$). The antibody response of infected chickens tested by an adeno-specific ELISA showed statistically significant ($P<0.001$) difference between the orally and im inoculated chickens.
The role of interleukins (IL) in the pathogenicity of and immune response to FAdVs is unknown. Therefore, in a chicken experiment IFN-γ, IL-10, IL-18 and IL-8 gene expression was evaluated following FAdV-4 infection. Cytokine gene expression was examined in the liver, spleen, and cecal tonsils. The dynamics of chicken cytokine expression revealed intriguing changes following FAdV-4 infection.

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Genetic characterization of Newcastle disease viruses, allocated in Ukraine in 2006-2009.

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Purpose: The purpose of this work was the characterization of Ukrainian field isolates of NDVs
Methods: Genetic material of NDVs, allocated in Ukraine (n = 8) was amplified in gene F partial fragment 374 bp under Aldous protocol. ABI-based sequencing was done and sequences were analysed with MEGA 4 in comparison with each genotype sequences. NJ rooted tree was constructed and its topology was analyzed.
Results: On the first stage of this study cDNA copies of viral RNA were amplified. As the result of PCR all NDV strains demonstrated amplicones formation. Purified products were analyzed. 2 isolates demonstrated low pathogenic cleavage site, and else 6 - highly pathogenic. Two visruses were alocated from domestic birds were characterized as genotype 2, strongly related to LaSota straine. Other agents were related to genotype 4 and 5 (4 and 2 respectively). Isolates from chicken were from genotype 5, and belonged to 5d genetic lineage. Other 4 isolates were allocated from pigeons (n = 3), and belonged to 4b (2) and 4d (1) genotypes, and from chicken (n = 1) - from 4b genotype. Divergence levels were 5-12 % in the group. Ukrainian strains demonstrated high levels of homology with Polish and other EU strains, and viruses allocated in arabian countries (97-100 %).
Conclusions: Subpopulations of NDVs circulating in Ukraine includes 2, 4 and 5th genotypes strains from chicken and pigeon origin, related to European and Asian strains

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Misfolded Y145stop catalyzes the conversion of full prion protein.

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Background: A point mutation in *Prnp* that converts tyrosine (Y) at position 145 into a stop codon leading to a truncated prion molecule in an inherited transmissible spongiform encephalopathy (TSE), Gertsmann-Sträussler-Scheincker syndrome, suggests that the N-terminus of the molecule (spanning amino acids 23-144) likely plays a critical role in prion misfolding as well as in protein-protein interactions.
Purpose: To provide a better understanding of prion misfolding, we investigated the role of the N-terminus of PRNP in conversion.
Methods and Results: Utilizing protein misfolding cyclic amplification (PMCA) we show that the recombinant polypeptide corresponding to the Y145Stop of sheep and deer PRNP can be *in vitro* converted in presence or absence of preexisting prions. In contrast, recombinant protein full-length PrP^C did not show a propensity for spontaneous conformational conversion to protease resistant isoforms. We found that seeded or spontaneously misfolded Y145Stop molecules can efficiently convert purified mammalian PrP^C into protease resistant isoforms. Furthermore, we show that prion seeding activity present in *invitro* converted Y145Stop triggered accumulation of protease-resistant prion protein in a transformed deer cell line (MDB).
Conclusions: These results establish that the N-terminus of PrP^C molecule corresponding to residues 23-144 plays a role in seeding and misfolding of mammalian prions.

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Pathogenicity and immune response in the lung of pigs experimentally infected with diverse genotype I PRRSV strains, including a pathogenic subtype 3 strain. **S.B. Morgan**[1], S.P. Graham[1], P.J. Sanchez-Cordon[2], F. Steinbach[1], J.-P. Frossard[1];
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Purpose: The evolution and diversification of porcine reproductive and respiratory syndrome viruses (PRRSV), particularly within genotype 1, is known to affect the clinical outcome of infection and poses significant problems in producing efficacious and broadly protective vaccines. The aim of this study is to characterise the in vivo response to a diverse range of genotype I PRRSV viruses.
Methods: Animals were inoculated with Lelystad virus (LV), a British field strain (215-06), both subtype 1 viruses, or a subtype 3 strain from Belarus (SU1-bel). Clinical scores and temperatures were monitored daily, post-mortems were performed at 3, 7 and 36 days post-infection (dpi), when lung gross pathology was scored and broncho-alveolar lavage fluid (BALF) was collected. Phenotypes of immune cell populations within the BALF were determined by flow cytometry. Virus load in BALF was determined using quantitative real-time PCR.
Results: Pigs infected with SU1-bel developed a fever and had significantly higher clinical scores compared with the other infected groups. Lungs from these pigs were found to have higher gross pathology scores at both 3 and 7 dpi. Virus load was higher in the lungs at 3 and 7 dpi for SU1-bel pigs compared to the 215-06 group, although surprisingly they were similar to pigs infected with LV. At day 7, there was an influx of immune cells into the lungs of infected pigs, which was most striking in the SU1-bel group. SU1-bel pigs presented higher numbers of CD8hi T cells and neutrophils compared with the others. Interestingly, only the LV and 215-06 infected groups displayed elevated numbers alveolar macrophages, with SU1-bel infected pigs having numbers comparable to that of uninfected controls.
Conclusions: This study describes the characterisation of the response to another pathogenic subtype 3 strain, highlighting the importance of this subtype and its potential impact on pig health in Europe. Experiments are on-going to test the hypotheses that (1) infiltration by neutrophils may explain the increased gross pathology seen following SU1-bel infection, and (2) the influx of cytotoxic lymphocytes into the lung may represent an important virus control mechanism.

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Sequence and virulence comparison of four North American isolates of porcine reproductive and respiratory syndrome virus.
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Purpose: Considerable genetic, antigenic and virulence differences exist among PRRSV isolates and depending on strain, dose and immune status, some farms may be subclinically infected with PRRSV while others experience severe reproductive and/or respiratory disease.
Methods: In this study, we compared the genomic sequence and virulence of 4 North American Type 2 PRRSV isolates, two pathogenic isolates that were associated with the emergence of novel and/or atypical PRRSV outbreaks in the late 1990's to early 2000's (SDSU73 and MN184) and two contemporary isolates of unknown pathogenicity and virulence (NADC30 and NADC31).
Results: Among the 4 isolates, SDSU73, MN184, and NADC30 were all clearly more virulent than NADC31, and among the 3 more virulent isolates, SDSU73 appeared to be the most virulent based on lung lesions, lymphadenopathy, febrile response, and decreased weight gains. Viral titers in the lung lavage and serum were highest for pigs infected with SDSU73 and MN184, and these were statistically greater than viral titers for pigs infected with the NADC31 isolate of PRRSV. Pigs infected with the NADC30 isolate had viral titers between those of pigs infected with either SDSU73 or MN184 and those of pigs infected with NADC31. Lesions consistent with bacterial bronchopneumonia were present to varying degrees only in pigs infected with PRRSV, and bacteria typically associated with the porcine respiratory disease complex were isolated from the lung of these pigs. Cytokine levels in the lung lavage were evaluated by multiplex ELISA and results show an increased production of several cytokines in pigs infected with PRRSV, but to varying degrees depending on the PRRSV isolate with which the pigs were infected. Genomic sequence evaluation indicates that both the NADC30 and NADC31 isolates of PRRSV maintain the nonstructural protein 2 deletion seen in MN184, but NADC31 has two additional 15 and 36 nucleotide deletions, and these strains are 8-14% different on a nucleotide basis from the MN184 strain.
Conclusions: Combined with the differing levels of attenuation, these results indicate specific genomic determinants of virulence are elusive and almost certainly complex.

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Isotype profile of PRRSV nucleocapsid-specific antibody response in pigs after experimental infection.
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Effective use of serology to determine the stage of PRRSV infection is one of challenges for prevention and control of PRRS. The objective of this study was to characterize kinetics of virus-specific IgG, IgM and IgA in pigs over time after experimental infection with VR2332 strain. Sera were collected from 27 pigs in 14-day intervals from 0 to 202 dpi and tested for various isotypes specific for viral nucleocapsid. The pigs comprised 4 groups based on the presence and absence of initial inoculation at 0 dpi and re-inoculation at193 dpi: A) inoculated/re-inoculated; B) inoculated/not re-inoculated; C) not inoculated/inoculated; and D) negative control. All samples were randomized first and, after 1:5 dilution, tested on IDEXX PRRS ELISA X3 kits using swine IgG-, IgM- or IgA-specific goat antibody labeled with HRP and TMB substrate. Optical density was measured at 450nm. Three sera with known isotype status and 2 kit controls were included in each plate to assess plate-to-plate variation. All samples and controls were run in duplicate. Group D stayed seronegative until 202 dpi. After inoculation, pigs developed IgG by 7-14 dpi. The IgG lasted at high level until 202 dpi and was not booted up by re-inoculation, suggesting that IgG is a good indicator of exposure. IgM appeared by 7 dpi and then rapidly declined. By 28 dpi, no IgM was detected. Re-inoculation did not elicit IgM response whereas group C developed IgM antibody sharply, indicating that IgM response can be indicative of first and recent exposure in naïve pigs. IgA appeared by 14 dpi, started to decline after 70 dpi to negative level by182 dpi, and was boosted up by re-inoculation, suggesting that IgA response may align better with host immune status to PRRSV. In conclusion, isotype-specific serologic assessment can be useful to determine infection and immune status of pigs.

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Novel PRRSV ORF5a protein is not immunoprotective but drives GP5 glycosylation.
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Introduction: Porcine reproductive and respiratory syndrome virus (PRRSV) is an enveloped RNA virus responsible for PRRS in swine; a disease with significant animal welfare and economic implications for which there is no specific treatment, and variable protection from vaccination due to viral genetic and antigenic diversity. Molecular mechanisms responsible for virulence, pathogenesis and protective immune response remain poorly understood. These

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factors limit progress towards development of effective measures for prevention and treatment of PRRS.
We have discovered a novel open reading frame (ORF) that is initiated upstream of and overlaps ORF5 encoding major envelope glycoprotein GP5. Presence of the ORF5a is evolutionarily conserved in all Arterivirus family members, and ORF5a protein is present in infected cells, incorporated into virions, and elicits antibody production in pigs infected with PRRSV.
Purpose: To investigate the paradox that ORF5a protein has a highly conserved arginine-glutamine (RQ) rich motif arising from nucleotide sequence dually encoding the GP5 hypervariable glycosylation domain that is assumed to be driven by immunological selection.
Methods: 4900 PRRSV sequences were examined to determine codon usage and infer selective pressures on this region.
Results: We determined that purifying selection to maintain ORF5a protein drives GP5 reading frame variation through selective ORF5a RQ codon usage.
Conclusions: This has implications for the variation in GP5 glycosylation pattern in this region where neutralizing epitopes have been described.
Purpose: To determine if ORF5a was immunoprotective.
Methods: Pigs were immunized with ORF5a protein prior to virulent virus infection.
Results: Immunized pigs had consistent serologic responses which were not immunodominant. Antibodies did not neutralize virus, and robust antibody responses observed in some pigs did not translate into protection against viral challenge as evaluated by viremia.
Conclusions: These findings indicate that ORF5a plays a role in viral biogenesis but does not elicit protective immunity.

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Development of virus-like particle vaccine of porcine reproductive and respiratory syndrome virus and analysis of immune responses in the vaccinated mice. **I.-S. Choi**[1], H.-M. Nam[1], Y.-J. Song[1], J.-B. Lee[1], S.-Y. Park[1], C.-S. Song[1], S.-M. Kang[2], M.-C. Kim[3];
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Purpose: The major problem of commercial PRRSV vaccines is that they provide a limited protection. Therefore, development of new vaccine having a potent protection efficacy is required. Virus-like particle (VLP) has been examined as a promising vaccine candidate for a variety of viral diseases because they induce both humoral and cellular immune responses. In this study, we developed a VLP vaccine composed of the GP5 and M of PRRSV and examined immune responses in mice vaccinated with it.
Methods: GP5 and M protein genes were amplified by RT-PCR and cloned into pFASTBAC. Both proteins were expressed in insect cells and the GP5-M VLP was purified using sucrose gradient. Female BALB/C mice, 5-6-week old, were divided into 7 groups with 10 mice in each group. The GP5-M VLP vaccine mixed with an adjuvant was administered to four groups of mice via intramuscular route with doses of 0.5 μg, 1 μg, 2 μg, and 4 μg, respectively. One group was immunized with 4 μg of the GP5-M VLP vaccine without adjuvant. PBS and a commercial killed PRRSV vaccine were injected to negative and positive control group, respectively. Mice were vaccinated three times at 2 weeks intervals and serum samples were collected before and after each vaccination. Mice were sacrificed at 2 weeks after third vaccination and splenocytes were collected. The antibody titers to GP5 were determined by indirect ELISA. The expression level of IFN-γ, IL-10 and IL-4 was determined in the antigen-stimulated splenocytes by ELISA and Real-time PCR.
Results: The identity of the GP5-M VLP was verified by Western blotting. A dose-dependent increase of the GP5-specific antibody was demonstrated in the vaccinated mice. IFN-γ and IL-10 were also significantly increased in a dose-dependent manner while IL-4 was moderately increased in all the vaccinated mice.
Conclusions: These results demonstrated that the PRRSV VLP could induce both humoral and cellular immune responses and be a new vaccine candidate for controlling PRRS.

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Intranasal delivery of an adjuvanted modified live porcine reproductive and respiratory syndrome virus vaccine reduces the ROS induced lung pathology.
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Reactive oxygen species (ROS) are produced predominantly by phagocytic cells in response to microbial infections. ROS when produced at optimal levels have potent antimicrobial properties. However, excess production of ROS induces apoptosis/necrosis of infected as well as bystander cells resulting in inflammatory pathology. Previously, we showed that vaccination of pigs with a modified live porcine reproductive and respiratory syndrome virus vaccine (PRRS-MLV) administered intranasally with a potent mucosal adjuvant *Mycobacterium tuberculosis* whole cell lysate (*Mtb* WCL) induces protective immune response against PRRS viral challenge. In this study, bronchoalveolar lavage fluid cells and peripheral blood mononuclear cells harvested from pigs vaccinated and challenged with PRRS virus were quantified for the levels of ROS production using colorimetric and flow cytometric analyses. Our results indicated that in vaccinated pigs (PRRS-MLV+*Mtb* WCL) levels of ROS were significantly less compared to unvaccinated PRRS virus challenged pigs. In control unvaccinated but PRRS virus challenged pigs the enhanced ROS production was associated with increased inflammatory lung pathology. In conclusion, our results suggested that intranasal vaccination using PRRS-MLV along with a potent mucosal adjuvant protects pigs against both homologous and virulent heterologous PRRS virus induced ROS mediated lung pathology. This project is supported by National Pork Board and USDA-NIFA PRRS CAP2 award to RJG.

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Effect of deoxynivalenol (DON) mycotoxin on porcine reproductive respiratory syndrome virus (PRRSV) *in vitro*.
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Deoxynivalenol (DON) is a mycotoxin produced by *fusarium spp*. DON should be treated as an important food safety issue because it is a very common contaminant of grains. Among monogastric farm animals, swine are the most susceptible to DON because it markedly reduces feed intake and decreases weight gain, even at low feed contamination. Investigations of host resistance, cell-mediated immune response and humoral immunity indicate that DON is both immunostimulatory and immunosuppressive depending on dose frequency and duration of exposure as well as type of functional immune assay.
The objective of this study was to investigate the *in vitro* effect of the DON mycotoxin on cythopathic effect and replication of PRRS virus in the permissive cell line MARC-145.
Non infected cells and cells infected with North American IAF-Klop PRRSV strain at 0.5 MOI, were treated with increasing concentration of DON mycotoxin (0, 70, 140, 280, 560, 1200 ng/ml). Cell survival was evaluated by determining the number of viable cells with a tetrazolium compound (CellTiter, promega) after 48 and 72hrs of infection. Virus titration was performed after 0, 24, 48 and 72 hrs of infection. Finally, cytokines mRNA

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expression was evaluated, after 24 and 48 hrs of infection, by qPCR.
DON significantly affects the survival of non infected cells at the concentration of 560 ng/ml or higher, but had no impact on cytokine mRNA expression of these cells. DON significantly increased the survival of cells infected with PRRS virus, at lower concentration, without affecting virus replication. However, high concentration of DON blocks virus replication, presumably by affecting cell survival. In PRRSV infected cells, low concentration of DON decreased cell expression of type-I interferon and TNF-α mRNA, but high concentration of DON increased mRNA expression of interferon-α but decreased mRNA expression of interferon-β and TNF-α.
The DON mycotoxin had a significant effect on the survival of PRRSV infected cells and on virus replication, in a dose dependant manner. For the moment, more experiment will be needed to determine the significance of these results, on PRRSV infection, *in vivo*.

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FISHing for cats: development of a fluorescence *in situ* hybridization (FISH) assay targeting feline papillomaviruses.
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Papillomaviruses (PVs) are ubiquitous host- and site-specific viruses. Their genomes comprise a circular molecule of double-stranded DNA, totaling approximately 8 kbp, coding for up to 11 open reading frames (ORFs). Currently, only six PV genomes of domestic and wild felid hosts have been isolated, cloned and fully sequenced. The link between the presence of feline papillomaviruses (FePVs) and clinical disease is presently difficult to assert with confidence due to the lack of an appropriate detection modality. However, evidence suggests that PV infections of cats are associated with cutaneous and oral papillomas, viral plaques, Bowenoid *in situ* carcinomas, squamous cell carcinomas and sarcoids. With increased reports of FePV-positive symptomatic cats in recent years, there is a need to investigate what role, if any, FePVs play in feline dermatoses. In this study, the development of a novel fluorescence *in situ* hybridization (FISH) assay was undertaken to determine if feline papillomavirus could be successfully visualized in tissues derived from suspect lesions from cats. FISH probes were generated from *Felis domesticus* papillomavirus type 2 (FdPV2) DNA obtained via PCR. Probes were labeled using digoxigenin then applied to formalin-fixed paraffin-embedded feline skin biopsies. Probe detection was facilitated via anti-digoxigenin Fab fragments conjugated with alkaline phosphatase (AP) and visualized with an AP-substrate fluorescent chromagen. Binding was interpreted as positive when confined to the nuclei of epidermal keratinocytes in suspect sections. Test cases were selected from feline lesions with previous histopathologic diagnoses of PV infection that had tested positive for FdPV2 by PCR (n=3). All test cases produced positive FISH results. This suggests that FISH can be a rapid and useful method for the detection of FePVs *in situ* and may provide a useful addition to current FePV diagnostic methodologies such as PCR or histopathology. This, in turn, may have positive implications in prognosticating patient outcomes and devising treatment strategies.

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Sequence analysis of swine influenza viruses circulating in US before and after the pandemic 2009 H1N1 influenza outbreak.
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Influenza A virus is a major respiratory pathogen of avian and mammalian species. The virus contains eight segments of negative-sense RNA (PB2, PB1, PA, HA, NP, NA, M, and NS), ranging from 2341nt to 890nt in size. Such segmentation of the genome allows influenza viruses to be reassorted with each other under certain conditions. Reassortment event can bring different genetic fragments of different origin into progeny viruses and can lead to antigenic shift. Nonetheless, most, if not all, of swine influenza viruses (SIVs) in US have internal genes (NP, M, and NS) of swine origin. In April 2009, a new H1N1 virus, now referred to as pandemic 2009 H1N1 (pH1N1), emerged in humans, which can also infect pigs. The pH1N1 virus has M gene of Eurasian lineage that has not been seen in North American SIVs. Thus, the M gene can be used as an indicator to study circulation of pH1N1 virus and reassortment event between endemic SIV and pH1N1 virus. In our study, the M gene of 121 H1N1 SIVs collected between 2006 and 2009 before pH1N1 outbreak were sequenced retrospectively. Sequencing for the M gene was also conducted on 54 SIVs collected after pH1N1 outbreak between 2010 and 2011. Selected isolates were sequenced for HA and NA genes when necessary. None of the 121 SIV isolates prior to pH1N emergence had M gene close to that of pH1N1 virus. Sequence identity with the prototype pH1N1 virus ranged from 86.8 to 88.4%, implying that pH1N1 virus was not originated from US pigs. Among the 54 isolates collected after pH1N1 emergence, 19 (35.2%) had M gene of pH1N1. Twelve of those also had HA and NA genes of pH1N1 virus while the remaining 7 SIVs contained HA and NA genes of endemic H1N1, H1N2 and H3N2 SIVs. Among the 35 isolates with M gene of endemic SIV, only one had HA and NA genes of pH1N1 virus. Our results clearly demonstrated that the pH1N1 virus has been circulating in US pig population after its emergence in human and a large scale of reassortment with endemic SIV has taken place. The rate of reassortment can be much higher than estimated in this study if other genes were to sequence. Clinical significance of reassortant viruses needs to be further studied.

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Identification of novel swine/pandemic H1N1 reassortant virus in pigs.
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In October and November 2010, novel H1N2 reassortant influenza viruses were identified from pigs showing mild respiratory signs that included cough and depression. The pooled oral fluids were used for PCR detection of influenza viral gene and virus isolation in Madin Darby canine kidney (MDCK) cell cultures. Sequence and phylogenetic analysis showed that the novel H1N2 reassortants possesses human-like HA and NA genes derived from recent H1N2 swine isolates similar to those isolated from Midwest and Southwest. The four internal genes, PB2, PB1, PA, and NS were similar to the contemporary swine triple reassortant viruses' internal genes (TRIG). Interestingly, NP and M genes of the novel reassortants were derived from the 2009 pandemic H1N1. The two isolates demonstrated one (E16G) and four (G34A, D53E, I109T, and V313I) unique amino acid changes in the M2 and NP proteins, respectively. The role of those amino acids in relation to host adaptation need to be further investigated. To our knowledge these isolate are the first human-like H1N2 virus isolated from pigs in the state of Ohio and the first swine and pandemic H1N1 reassortant identified in the U.S. The reassortments of pandemic H1N1 with swine influenza viruses indicate the importance of systematic surveillance of swine population to determine the origin, the prevalence of similar reassortants in the U.S. and their impact on both swine production and public health.

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In vitro reassortment between endemic H1N2 and pandemic 2009 H1N1 Swine Influenza Viruses

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The pandemic H1N1 (pH1N1) influenza was first reported in humans in the spring of 2009 and soon thereafter was identified in numerous animal species, including swine. Reassortant viruses, presumably arising from the co-infection of pH1N1 and endemic swine influenza virus (SIV), were subsequently identified in diagnostic samples from swine in North America. While the genetic constellation of the reassortant viruses was diverse, the combination of hemagglutinin (HA) and neuraminidase (NA) genes of endemic SIV origin and the matrix gene from pH1N1, was conserved. In this study, co-infection of swine testicle cells with swine-derived endemic H1N2 and pH1N1 yielded two reassortant H1N2 viruses, both possessing a matrix gene derived from pH1N1, HA and NA genes from the endemic H1N2 virus, and different constellations of internal genes derived from the H1N2 and pH1N1 viruses. In cell culture, the reassortant viruses reached titers similar to the H1N2 parent and approximately 2 $\log_{10}$ $TCID_{50}$/mL higher than the pH1N1 parent. In addition, the Eurasian lineage M and NA genes from pH1N1 were inserted into a triple reassortant internal gene cassette (TRIG) H1N1 SIV using reverse genetics, both individually and in combination. Growth kinetic studies found little differences in viral titers, further indicating the genetic compatibility between endemic and pH1N1 viruses. This *in vitro* work demonstrates for the first time that endemic and pH1N1 viruses, circulating in North American swine herds, can undergo genetic reassortment and generate viruses with improved growth kinetics.

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Evaluation of cd25, foxp3, and ccl5 gene expression in placentomes of pregnant cattle inoculated with bovine viral diarrhea virus.

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Purpose: CD4+CD25+ T regulatory cells have been associated with immunosuppressive activity in the pregnant uterus of humans and some animal species, playing a significant role in maintaining pregnancy by suppressing placental inflammation. We have observed minimal inflammation during microscopic examination of bovine placentas following intrauterine infection with bovine viral diarrhea virus (BVDV), a Pestivirus which crosses the placenta with high efficiency. The objectives of this study were to evaluate if BVDV is recognized in the placenta by the local maternal immune system, despite the absence of significant inflammatory lesions. Additionally, we wanted to characterize the immune response to bvdv at the maternal-fetal interface and evaluate potential mechanisms by which BVDV alters normal local immunoregulatory processes at this site.
Methods: Heifers naive to BVDV were synchronized, bred, and upon confirmation of pregnancy were divided into principal (n=11) and control groups (n=11). The principal group was inoculated intranasally with $1.0x10^5$ $TCID_{50}$/ml of the type-2 strain PA131 between gestational days 89 to 110. Randomly selected samples from the uterus, placenta, and fetal tissues were collected between 150-157 days gestation. Total RNA was extracted from placentomes and prepared for real-time reverse transcription PCR to quantify gene expression. Results: Expression of CD25, FOXP3, and CCL5 in placentomes of principal, control groups, and immune tissue controls (mesenteric lymph nodes) was observed. Following normalization of target genes with housekeeping genes, FOXP3 and CCL5 were expressed at higher levels in placentomes of heifers infected with BVDV than control heifers. Conclusions: Detection of CD25, FOXP3 and CCL5 in the bovine placenta has not been documented previously and provides a baseline for characterization of the immunological profile of the normal placenta at 150 days gestation. These findings suggest there is immune recognition of the virus at the maternal-fetal interface and the virus may alter normal expression of T regulatory cell markers.

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PCV2 infection from birth through finishing.

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Porcine circovirus 2 (PCV2) infection, the causative agent for PCV2-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 PCV2 levels in serum, but does not eliminate infection. Improving vaccine efficacy against infection requires a better understanding of when and how pigs are exposed to and become productively infected with PCV2. Previously, infection was thought to occur in finishers at 10-15 weeks of age, when they become viremic. However, PCV2 has been shown to be present in sows and finishers, as well as shed in colostrum, milk, and feces, and is stable in the environment. Our previous research has shown that PCV2 can be found in serum from sows, colostrum, oral fluids, feces, and the farrowing environment, suggesting PCV2 is everywhere. PCV2 was also observed in presuckling piglet serum and stillborn and mummified fetuses, suggesting that animals are infected with PCV2 in utero. PCV2-specific antibodies were also observed at high levels in sow serum and colostrum. These data suggests that piglets are exposed to PCV2 in utero, as well as constantly challenged by the virus after birth, but antibodies from the sow may be able to control infection. We hypothesize that piglets are infected with PCV2 in utero, but colostral antibodies suppress viremic infection until the antibodies decreases at 10-15 weeks of age, at which time viremia becomes evident. We sampled piglet serum from birth (presuckling) through finishing (3, 10, 15, and 24 weeks of age) for PCV2 DNA and PCV2-specific antibodies. Animals were sacrificed at each time point and tissues were examined for PCV2 DNA. Virus was sequenced throughout the time points to determine if the infecting viral isolate changed during the life of the piglets. Presence of live virus was confirmed by growth in cell culture. 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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