PROGRAM & PROCEEDINGS

96th Annual Meeting of the Conference of Research Workers in Animal Diseases

December 6, 7 and 8, 2015

Marriott, Downtown Magnificent Mile

Chicago, IL

The 96th Annual meeting of the CRWAD is dedicated to

Dr. Prem S. Paul

David A. Benfield, Executive Director and Editor CRWAD Program

Abstracts Available at the On-Line Meeting Planner and Itinerary Builder

www.crwad.org

Proceedings Distributed by CRWAD

CRWAD 96th ANNUAL MEETING – 2015

December 6-8, 2015

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

Registration – 5th Floor Registration Desk

Sunday December 6 10 AM – 5 PM
Monday December7 7 AM – 5:30 PM
Tuesday December 8 8AM – 11 AM

Researchers Reception – Welcome to all attendees – casual wear

Sunday December 6, 6-8 PM Grand Ballroom III – 7th Floor
Introduction of Dedicatee, CRWAD Council, New members

Student Reception Students and invited guests 5:00 – 5:45 Grand Ballroom III – 5th Floor

CRWAD Business Meeting – Salons A/B/C/D – 5th Floor

Tuesday December 8, 11:45 AM – 12:30 PM
Dedication of the meeting, business, and awards
All attendees are invited to attend

Speaker Ready Room in Streeterville Room 2nd Floor, Sunday December 6- Monday December 7.

Meeting Session	Monday AM 8:00-11:30 Room & Floor	Monday PM 1:30-4:30 Room and Floor	Tuesday AM 8:00-11:30 Room and Floor
Bacterial Pathogenesis	Avenue Ballroom 4th Floor	Avenue Ballroom 4th Floor	
Biosafety and Biosecurity		Northwestern/Ohio 6th Floor	
Companion Animal Epidemiology	Salon E – 5th Floor		
Ecology and Management of Foodborne Agents		Salon E – 5th Floor	Salon A/B/C/D 5th Floor (join Epidemiology for keynote and Mark Gearhart award)
Epidemiology and Animal Health Economics	Salons A/B/C/D 5th Floor	Salons A/B/C/D 5th Floor	Salons A/B/C/D 5th Floor
Immunology	Salons F/G/H 5th Floor	Salons F/G/H 5th Floor	Salons F/G/H 5th Floor
Pathobiology of Enteric and Foodborne Pathogens	Michigan/Michigan State 6th Floor	Michigan/Michigan State 6th Floor	Michigan/Michigan State 6th Floor
Respiratory Diseases	Indiana/Iowa 6th Floor	Indiana/Iowa 6th Floor	
Vector-Borne and Parasitic Diseases	Denver/Houston 5th Floor	Denver/Houston 5th Floor	
Viral Pathogenesis	Los Angeles/Miami 5th Floor	Los Angeles/Miami 5th Floor	Los Angeles/Miami 5th Floor
Posters in Grand Ballroom	Salon III – 7th Floor Sunday 6:00- 8:00 PM	Salon III – 7th Floor Monday 5-6:30 PM	

Sunday Poster Session. Posters boards available for poster assembly at 4 PM Sunday: Bacterial Pathogenesis: Companion Animal Epidemiology; Epidemiology and Animal Health Economics; and Pathobiology of Enteric and Foodborne Pathogens, Poster session from 6:30 – 8:00 PM. Please remove posters by 10:00 AM Monday. **Poster presenters should furnish their own tacks.**

Monday Poster Session. Poster boards will be available for post assembly by noon on Monday. Ecology and Management of Foodborne Agents; Immunology; Respiratory Diseases; Vector-Borne and Parasitic Diseases; and Virology. Presentations will be from 5:00 – 6:30 PM. Please remove posters by 6:30 PM. **Poster presenters should furnish their own tacks.**

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 height and 8 ft width.

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2016 CRWAD Meeting information	Outside back cover

2015 CRWAD Registration Information and Information for Speakers and Poster Presentations

All participants at the CRWAD meeting must be registered and have a name badge to be admitted to sessions and other events

CRWAD Registration – 5th Floor Foyer Registration Desk

Sunday December 6, 10 AM – 6 PM
Monday December 7, 7 AM – Noon, 2 -5 PM
Tuesday December 8, 8 AM – 11 AM

CRWAD Researchers Reception and Poster Session I – Grand Ballroom Salon I- 7th Floor Sunday December 6

Poster Session I setup, 4 PM (remove posters by 10 AM Monday morning). Posters for Bacterial Pathogenesis, Companion Animal epidemiology, epidemiology and Animal Health Economics and Pathobiology of Enteric and Foodborne Pathogens. There will also be posters from the North American PRRS Symposium.

Poster Session I, 6:30-8 PM

Researchers Reception and Recognition of New CRWAD members, 6 PM, reception ends 8 PM.

All attendees' welcome, casual wear recommended

CRWAD Poster Session II – Grand Ballroom Salon 1, 7th Floor Monday December 7

Poster Session II setup, 12:00 PM (remove poster by 6:30 PM).
Posters for Ecology and management of Foodborne Agents, Immunology, Respiratory Diseases, and Virology.
Poster Session II, 5 – 6:30 PM

Note to Poster Presenters. You must be with your competition entry posters for possible judge interviews on Sunday and Monday.

Award winners are recognized at the CRWAD Business Meeting on Tuesday December 8, 12:45 – 1:30 PM. Please plan to attend.

CRWAD Students and Post-Docs Reception

Sunday, Grand Ballroom Salon 1, 7th Floor, 5-6 PM
All full time students, post docs, CRWAD Council Members, Dedicatee, Keynotes and other invited guests.

Speaker Ready Room

Streeterville Room, 2nd Floor available Sunday Dec 6 – Monday Dec 7.

CRWAD Business Meeting

Tuesday December 8, 12:45 – 1:30 PM. Recognition of Distinguished Veterinary Immunologist and Microbiologist, Schwabe Award in Epidemiology and various graduate student awards for best oral and poster presentations. Plan to attend and cheer on you institutional winners.

CRWAD THANKS OUR 2015 SPONSORS

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Bronze Medal Contributor $1000.00

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USDA United States Department of Agriculture National Institute of Food and Agriculture

The CRWAD Conference is supported by the National Institute for Food and Agriculture (NIFA) grant 2015-06990 of the USDA Agriculture and Food Research Initiative (AFRI) two programs: AFRI Foundational Animal Health and Disease and AFRI Foundational Food Safety. USDA NIFA is also supporting in collaboration with CRWAD travel awards to faculty from 1890 Land Grant Universities.

North American PRRS 2015 Symposium

CRWAD Appreciates a contribution from MVP Corporation.

2015 CRWAD Tabletop Exhibitors

Please visit the Exhibitors Displays and Thank Them for Supporting CRWAD

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

http://journals.cambridge.org/action/displayJournal?jd+AHR

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

www.elsevier.com/anivet

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

www.genereach-us-com

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

http://www.listabs.com/

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

www.mabtech.com

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

www.perkinelmer.com

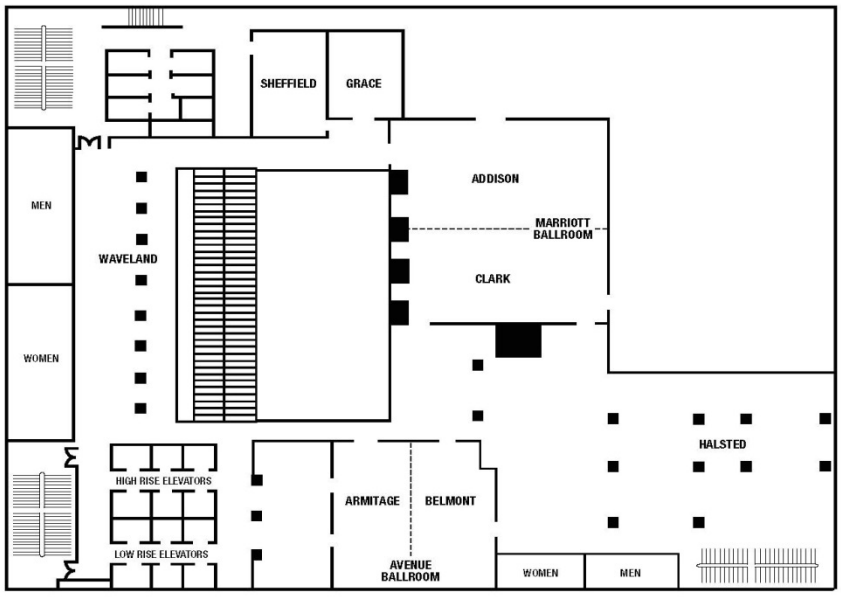
Qiagen. A provider of sample and assay technologies for molecular diagnostics, applied testing, academic and pharmaceutical research. Consolidated under the Dutch holding Qiagen, NY, the company operates more than 35 offices in over 20 countries.

http://www.qiagen.com/

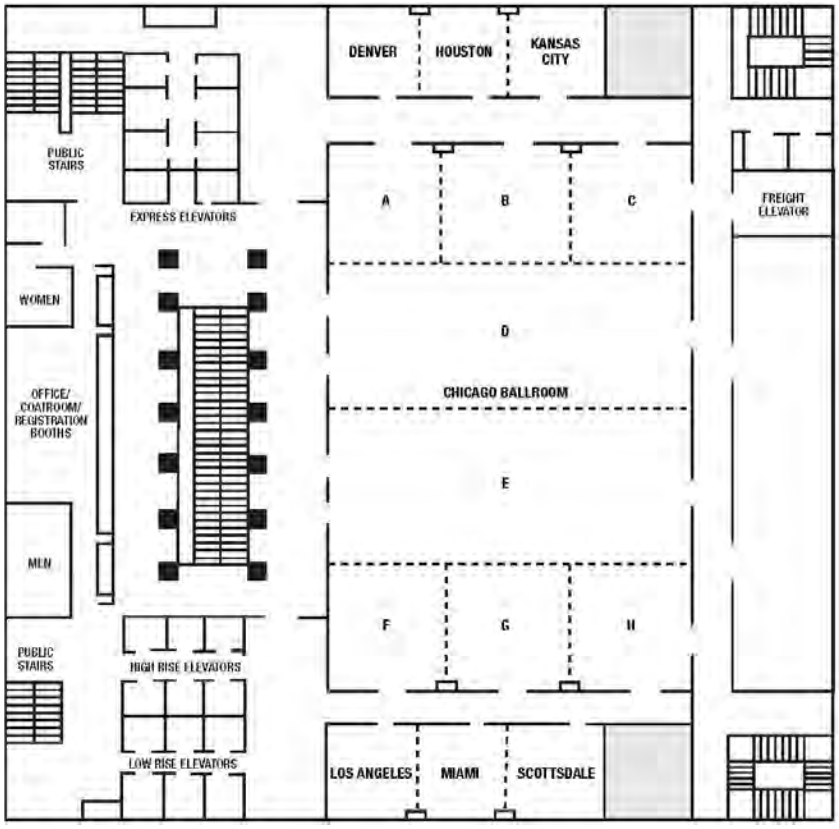
Tetracore, Inc. An industry leader in the development of rapid tests for agricultural animal diseases. Tetracore's dried qPCR tests are ideal for surveillance monitoring and the EX-PRRSV MPX 4.0 reagents are the industry gold standard for high throughput PRRSV detection. The T-COR 4 instrument allows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

www.tetracore.com

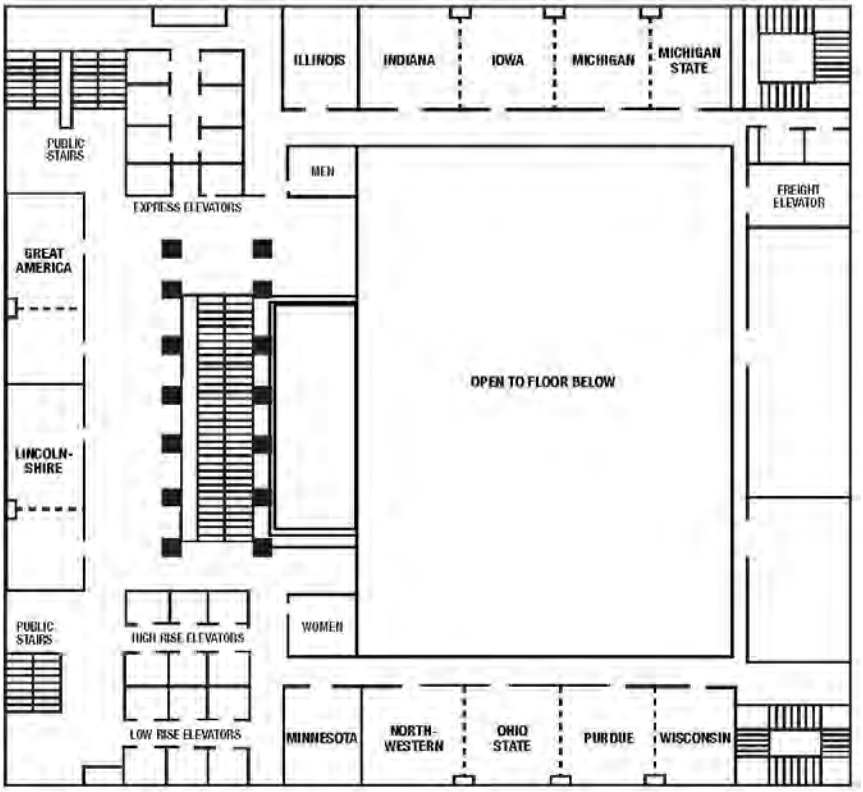
Chicago Marriott Floor Plan 4th Floor



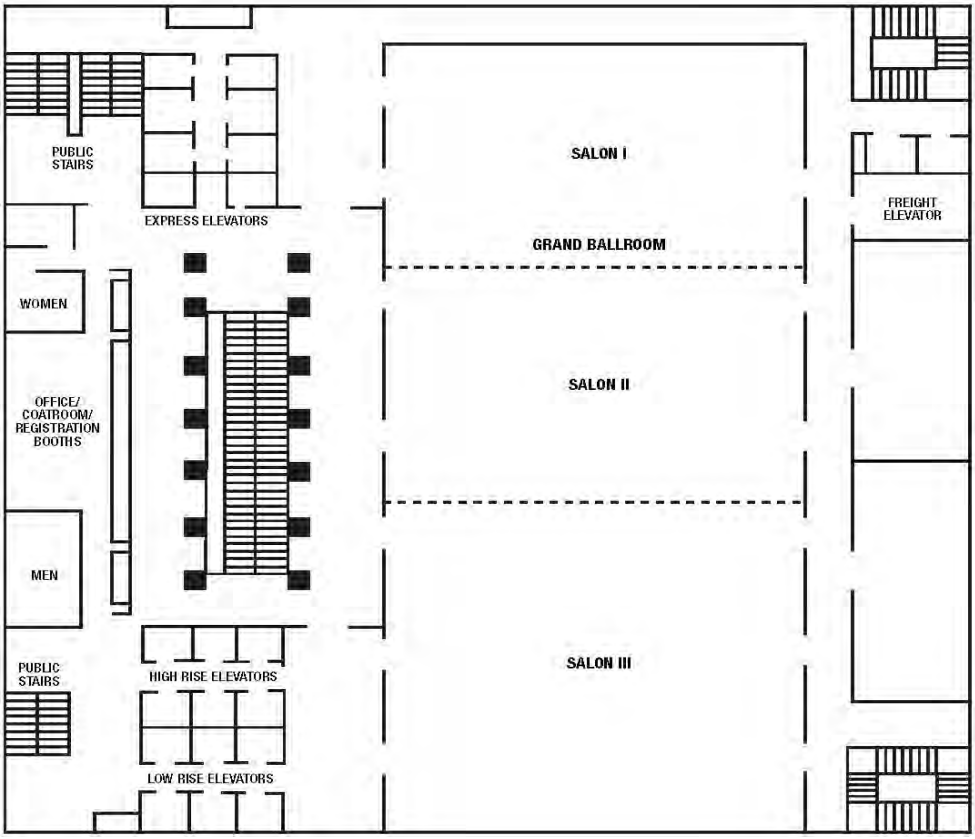
Chicago Marriott Floor Plan 5th Floor



Chicago Marriott Floor Plan 6th Floor



Chicago Marriott Floor Plan 7th Floor



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The Conference of Research Workers in Animal Diseases

The Conference of Research Workers in Animal Diseases (CRWAD) was established in 1920 and has been a non-profit organization since that time. The purpose of CRWAD is to provide a venue for the discussion and dissemination of the most current research advances on diseases of companion and agricultural animals. Faculty, post-doctoral research associates, graduate students and industry scientists present and discuss new and innovative information on the epidemiology, immunology, epidemiology, vector-borne and parasitic diseases, food borne pathogens, viral and bacterial diseases and biosafety and biosecurity. We encourage all to review qualifications to become a member of CRWAD at the website http://www.crwad.org and to be a member of the most exclusive group in the world on animal diseases.

The annual meeting is held on a Sunday through Tuesday format in early December. There are 10 concurrent scientific sessions and two poster sessions held at the meeting. Nearly 400 scientists attend this annual gathering of animal disease experts. Highlights of the meeting are the recognition of an outstanding individual in the field of animal diseases as the Dedicatee of the CRWAD meeting. Also many group sponsor graduate student awards for the best oral and poster presentations.

All oral and poster Abstracts are published in an annual CRWAD Proceedings that are available for purchase at the meeting. Abstract are also available at the on-line meeting planner and itinerary builder http://www.crwad.org.

Thank you for attending the annual meeting. If you have questions related to the meeting or the organization please contact Dr. David A. Benfield, Executive Director at benfield.2@osu.edu or Loren Harper, Administrative Assistant, harper.202@osu.edu.

2015 CRWAD Council

Roman Ganta, Kansas State University, President
Laurel Gershwin, University of California-Davis, Vice President
Paul S. Morley, Colorado State University, 2011-2015
Christopher Chase, South Dakota State University, 2012-2016
Qijing Zhang, Iowa State University, 2013-2017
Amelia Wollums, Mississippi State University, 2014-2018

Executive Director: David A. Benfield, Ohio State University

Administrative Assistant: Loren D. Harper, Ohio State University

2015 CRWAD Dedicatee
Prem S. Paul, D.V.M., Ph.D.

Dr. Prem Paul's career began in Hissar, Haryana, India as a veterinary medicine student. After receiving his DVM (BVSc) from the Panjab Agricultural University College of Veterinary Sciences in 1969, he moved to the United States to pursue a PhD in veterinary microbiology at the University of Minnesota in St. Paul, Minnesota. He received his PhD in 1975, and in 1977 became a diplomate in the American College of Veterinary Microbiologists.

Dr. Paul's research career began at the University of Minnesota as a research associate in the Department of Large Animal Clinical Sciences. He moved to Ames, Iowa, and served as the veterinary medical officer at the USDA National Animal Disease Center for seven years. In 1987, Dr. Paul joined the faculty of Iowa State University as a member of the Veterinary Medical Research Institute. During his time at ISU he served as director of graduate education (1991-1992), professor-in-charge (1992-1993) and associate director (1993-1999) of the Veterinary Medical Research Institute; assistant director of the Iowa Agricultural and Home Economics Experiment Station (1996-2000); associate dean for research and graduate studies for the College of Veterinary Medicine (1993-1999); and associate vice provost for research (2000-2001). In 2001, Dr. Paul accepted the position of dean of graduate studies and vice chancellor of research at the University of Nebraska–Lincoln in Lincoln, Nebraska. His title changed in 2008 to vice chancellor for research and economic development to reflect new responsibilities.

Dr. Paul's scientific expertise is in animal virology. He began his career working with poultry viruses such as HVT, Marek's disease virus and reticuloendotheliosis virus. He has made significant contributions to animal health through research on viral pathogenesis of respiratory and reproductive diseases, leading to improved vaccines and diagnostic tests.

Dr. Paul has been extremely proactive in advancing student exposure to research. He was instrumental in developing the Merial Veterinary Scholars Program, a national program that funds student research at veterinary schools and hosts a national symposium where students present their findings.

His impact on animal health/disease research can be measured in part by the accomplishments of his former students, which include a number of nationally and internationally renowned

scientists at educational institutions and with government agencies in the United States and abroad.

He is a Charter Fellow of the National Academy of Inventers and a fellow of the American Association for the Advancement of Science. He has served on review panels for NIH, the USDA and the NSF and was a member of the U.S. Food and Drug Administration's xenotransplantation advisory subcommittee. He is past chair of the EPSCoR Coalition Board and the Council on Research Policy and Graduate Education. He was a member of the National Academies Committee on Policy Implications of International Graduate Students and Postdoctoral Scholars in the United States, and past president of the Council of the Conference of Research Workers in Animal Diseases.

He is a member of the American Veterinary Medical Association, American Association for the Advancement of Science, American College of Veterinary Microbiologists, American Society for Virology, Conference of Research Workers in Animal Diseases, and the American Association of Swine Veterinarians.

He has authored more than 100 papers in refereed publications, has edited two books, 11 book chapters and holds more than 20 U.S. and international patents.

Dr. Paul and his wife Melissa (Missy) have two children, Neena who resides in New York City and Ryan who lives in Chicago. The Pauls have one granddaughter.

Recent Past Presidents CRWAD

David A Benfield	2014	Rodney Moxley	2013
Donald L Reynolds	2012	Laura L Hungerford	2011
Eileen L Thacker	2010	Bill Stich	2009
Richard E Isaacson	2008	Lynn A Joens	2007
Prem S Paul	2006	Ian Gardner	2005
Janet MacInnes	2004	Katherine M Kocan	2003
Franklin A Ahrens	2002	Linda J Saif	2001
Leon ND Potgieter	2000	MD Salman	1999
Donald G Simmons	1998	Bert E Stromberg	1997
Patricia E Shewen	1996	Bradford B Smith	1995
Ronald d Schultz	1994	Lawrence H Arp	1993
Richard F Ross	1992	Robert M Corwin	1991
Lynette B Corbeil	1990	William C Wagner	1989

The Dedicatee Tradition

Annually, CRWAD selects a Life Member who has made outstanding contributions to CRWAD and to animal disease research to be honored as the Dedicatee for the CRWAD meeting. The tradition started in 1974 and continues to the present. The Dedicatee is honored with a plaque and an hororarium at the annual business meeting. All recognized Dedicatees are listed below.

WR Hinshaw	1974	SH McNutt	1975	HCH Kernkamp	1976
RW Doughtery	1977	CH Bradley	1978	SFScheidy	1979
AG Karlson	1980	IA merchant	1981	LC Ferguson	1982
F Maurer	1983	C Olson, Jr	1984	C Cunningham	1985
BS Pomeroy	1986	N Levine	1987	E Splitter	1988
MJ Twiehaus	1989	RA Packer	1990	DA Barnum	1991
AF Weber	1992	EO Halterman	1993	EM Kohler	1994
EH Bohl	1995	LE Hanson	1996	GR Carter	1997
JB Derbyshire	1998	BC Easterday	1999	L Coggins	2000
DP Anderson	2001	J Storz	2002	AJ Winter	2003
HW Moon	2004	WJ Mengeling	2005	LE Carmichael	2006
RF Ross	2007	SA Ewing	2008	NF Cheville	2009
SK Maheswaran	2010	DO Simmons	2011	WC Wagner	2012
FW Scott	2013	DC Robertson	2014	PS Paul	2015

In Memoriam - Richard E. Corstvet, 1928 – 2014

Richard E. Corstvet died October 18, 2014. For many years he was a fixture at Conference of Research Workers in Animal Disease (CRWAD) meetings each autumn. He, his graduate students and other colleagues contributed regularly to the scientific program.

Corstvet was dedicated to the well-being of students, both veterinary medical and graduate. The year after he left Oklahoma State University (OSU) for Louisiana State University (LSU), the OSU student yearbook, the *Aesculapius*, devoted a full page to him, including two photographs. The page carried the following message (1982, p. 55):

"IN RECOGNITION OF … a man admired and respected by both students and faculty. He has never been a politician, nor has he ever attempted to be anything but himself. He has always been willing to stand up for what he feels is right, always deeply concerned with the quality of the students' veterinary educational experience. It is with profound appreciation and affection that we recognize Dr. Richard E. Corstvet."

The students' reference to his not being a "politician" probably derived from his reputation for "calling a spade a spade" in whatever setting he found himself. He could be abrupt, but those who knew him never questioned his dedication to helping others. He wanted students to succeed, and he was consistent in helping younger faculty colleagues in their professional development.

A native of Wisconsin, Corstvet received his undergraduate and first graduate degree from the University of Wisconsin – Madison in 1951 and 1955, respectively. He was awarded the Ph.D. in veterinary microbiology in 1965 by the University of California – Davis.

While in California, Corstvet focused primarily on avian diseases. He published extensively on *Mycoplasma* spp., erysipelas, and Newcastle disease, among others.

Corstvet joined Oklahoma State University in 1965 and rose through the academic ranks to professor. His success as an educator is attested to above by the tribute from OSU veterinary students. His success in research is documented in the peer-reviewed literature. Among many disease processes he studied, his contributions to bovine respiratory disease (BRD) stands out. He and Roger Panciera developed a model for testing the immune response of cattle to vaccines aimed at protecting cattle from BRD.

After moving to LSU, Corstvet organized the microbiology diagnostic service for the then-relatively-new program. He and colleagues at LSU collaborated on studies of respiratory and reproductive diseases of cattle. Among other research interests, he undertook studies of canine ehrlichiosis. He and four colleagues received a patent for a vaccine against *Ehrlichia canis*.

Sidney A. Ewing
Oklahoma State University

PROGRAM

2015 CRWAD Satellite Meetings and Programs

USDA NIFA Project Directors/Principle Investigators Meeting
Friday December 4, Salon 1, 7th Floor, 8 AM – 5 PM.

American College of Veterinary Microbiologists (ACVM) Examinations
Friday December 4, Denver/Houston Room, 5th Floor, 12 PM – 5 PM
Saturday December 5, Denver/Houston Room, 5th Floor, 8 AM – 9 PM
Saturday December 5, Kansas City Room, 5th Floor, 8 AM – 1 PM
Sunday December 6, Indiana/Iowa Room – 5th Floor
Examination Committee Meeting 8 - 9 AM
Board of Governors 9AM – 12 PM
Attendance by invitation only

American Association of Veterinary Immunologists (AAVI) Board Meeting
Sunday December 6, Indiana/Iowa Room – 5th Floor, 1 – 5 PM.

Animal Health Research Reviews (AHRR) Board Meeting
Tuesday December 8, Grace Room – 4th Floor 7 – 8:30 AM
Section Editors and Editorial Board joint meeting, contact Bill Stich, Editor in Chief for more information

Association for Veterinary Epidemiology and Preventive Medicine (AVEPM) Business Meeting – Members only
Monday December 7, 11:30 – 1:30 PM – Salon A/B/C/D - 5th Floor

CRWAD Council Meeting – Council members only
Saturday December 6, Great America Room – 6th Floor 5:30 – 8 PM

CRWAD Sponsorship Committee Meeting (report to CRWAD Council)
Saturday December 6, Great America Room – 6th Floor, 5:30 – 6 PM

Distinguished Veterinary Immunologist Lecture by Dr. Phillip Griebel
Monday December 7 Salons E/F/G – 5th Floor 1:30 PM Immunology

Distinguished Veterinary Microbiologist Lecture by Dr. John Prescott
Monday December 7 Avenue Room – 4th Floor 10:45 AM Bacterial Pathogenesis

NC 1202 Enteric Diseases of Food Animals: Enhanced Prevention, Control and Food Safety
Saturday December 6, Lincolnshire Room 8 AM – 5 PM
Sunday December 7, Lincolnshire Room – 5th Floor 8 AM – 12 PM
Attendance by invitation only. Contact Weiping Zhang (wpzhang@vet.k-state.edu

Workshops, Symposia and Mini-symposia

AAVI mini symposium on Mucosal Immunology
Monday afternoon session in the Immunology section, Salons F?G?H – 5th Floor 1;30 – 4:30, contact Renukaradhya Gourapura or Radhey Kaushik.

ACVM Mini-symposium on Clostridia and Associated Diseases
ACVM Distinguished Microbiologist presentation, Avenue Room – 4th Floor Dr. John Prescott, Monday December 7, 10:45 AM
Mini-symposium Monday afternoon session in the Bacterial Pathogenesis section, Avenue room – 4th Floor, 1:30 – 4:30 PM.

AVEPM Workshop – Epidemiology Educators Forum
Sunday December 6, Salons E/F/G/H – 5th Floor, All attendees welcome, Contact Brandy Burgess burgessb@vt.edu

AVEPM Schwabe Symposium – Big Open, Crowd-sourced and Exhaust(ed)! What opportunities do these data sources hold for Veterinary Epidemiology?
Sunday December 6, Salons E/F/G/H – 5th Floor, 12:30 PM – 5:00 PM.
All attendees welcome. Contact Julie Funk junkj@cvm.msu.edu

International Brucellosis Society Meeting
Saturday December 5, Salon D – 5th Floor, 8:00AM – 5:00PM
Sunday, December 6, Salon D – 5th Floor, 8:00AM – 12:00PM
Contact Sue Hagius shagius@agcenter.lsu.edu

NC 229 Detection and Control of Porcine Reproductive and Respiratory Syndrome Virus and Emerging Viral Diseases of Swine, Mini symposium on emerging and other swine diseases, continuation of North American PRRS Symposium.
Sunday December 6 Northwestern/Ohio 1:00 – 5:30 PM
Members and attendees invited, contact Fernando Osorio (fosorio.1@unl.edu) or KJ Yoon (kyoon@iastate.edu)

2015 Schwabe Symposium:

Big, Open, Crowd-sourced and Exhaust(ed)! What opportunities do these data sources hold for Veterinary Epidemiology?

Abstract: Evolution in technology has made the possibilities for data collection, information-exchange, networking, and data integration limitless. Using new and emerging technologies to conduct research, promote animal or human health behavior change and facilitate decision making is fast becoming the norm. Grassroots communication efforts have stimulated technological innovations that are facilitating social change (e.g., Crisis Commons and Ushahidi), capturing epidemiological trends (e.g., Google Flu; Bernardo et al., 2013), driving the development of the so-called 'quantified self' and transforming the nature of human and animal health systems (e.g., Patients Like Me, I-Cow, LifeLearn Sofie).

What do these technological advances mean for veterinary epidemiology? What is the impact on our methodologies? How may they impact our pedagogy? Are they transformational (or disrupting) forces in epidemiological research and veterinary medicine?

We have invited a prestigious group of speakers to share models and insight for the use of emerging technologies for research, public and animal health, and clinical practice. A key aspect of the symposium is a panel question and answer session on how these novel data sources impact research, teaching and practice in veterinary medicine and epidemiology.

Agenda:

12:30 Introduction: Dr. Julie Funk, Michigan State University

12:45 The Epinet: Implications of connected beings and things, *Dr. Theresa Bernardo, Professor and IDEXX Chair in Emerging Technologies and Bond-Centered Animal Health Care, Ontario Veterinary College, University of Guelph*

1:30 Artificial intelligence in animal health: case study in Sofie, veterinary medicine's first IBM Watson solution.(http://www.lifelearn.com/innovations/lifelearn-sofie/)' , *Dr. Adam Little, Director, Partnerships and Innovation, LifeLearn, Inc., Guelph, Ontario, Canada*

2:15 Coffee Break

3:00 Crowd-sourcing public health: The Foodborne Chicago Project (https://www.foodbornechicago.org/) , *Daniel X. O'Neil, SmartChicago Collaborative, Chicago, IL*

3:45 Big Data for small things: Metagenomic approaches to understanding antimicrobial resistance, *Noelle R. Noyes, USDA NIFA Postdoctoral Fellow., Colorado State University*

4:30 Panel Discussion: Participation by all speakers.

Sun, Dec 6, 2015
12:30-5:00 PM
Chicago Ballroom, E/F/G/H 5th Floor

Presented by the Association for Veterinary Epidemiology and Preventive Medicine

Made possible by the
AVEPM Continuing Education Committee
and a generous gift from
Bayer Animal Health

2015 CRWAD – Keynote Speaker – Immunology Session AAVI Distinguished Veterinary Immunologist Award

Philip J. Griebel, DVM, PhD

School of Public Health and VIDO-Intervac, University of Saskatchewan, Saskatoon, SK, Canada
Abstract No. 306 – Title: First encounters: Mucosal immune system development and the microbiome.

Monday, December 7, 2015 1:30PM Salon F,G,H – 5th Floor

Philip J. Griebel, University of Saskatchewan, Saskatchewan, SK, Canada

Philip Griebel is a Research Fellow at the Vaccine and Infectious Disease Organization (VIDO-Intervac) and Professor in the School of Public Health at the University of Saskatchewan (U of S), Saskatoon, SK, Canada. He graduated with a BSc in Cell Biology from the University of Victoria in 1977, obtained a DVM from the Western College of Veterinary Medicine, U of S, in 1981, and subsequently completed a PhD in Immunology at the U of S in 1988. He has been active in research related to bovine mucosal immunity and vaccines for over 25 years and has published over 150 peer-reviewed articles. His current research focus is development of the mucosal immune system in newborn calves. This research includes developing new vaccine technologies and strategies to enhance disease protection, characterizing the role of the commensal microbiome in mucosal immune development, and analyzing the role of stress in infectious disease.

2015 CRWAD – Keynote Speaker – Bacterial Pathogenesis Section and ACVM Distinguished Veterinary Microbiologist

John F. Prescott

Department of Pathobiology, University of Guelph, Guelph, Ontario N1G 2W1, Canada
Abstract No. 331 – Title - Driving through the fog: Understanding type A *Clostridium perfringens* in enteric disease of animals

Monday, December 7, 2015, 10:45AM – Avenue Ballroom – 4th floor

John F. Prescott is a retired University Professor Emeritus from the University of Guelph and 35-year member of the CRWAD. He has diverse interests in bacterial infections in animals, including leptospirosis, but is best known for work on *Rhodococcus equi* pneumonia in foals and in promoting better use of antimicrobial drug use in animals. He is an editor and an author of the text "Antimicrobial Therapy in Veterinary Medicine", now in its fifth edition. A Chinese language edition is in the works. He has helped to organize 3 national conferences on antimicrobial use and resistance in animals in Canada, and is active in antimicrobial stewardship advocacy. More recent research interests are on immunization against necrotic enteritis of broiler chickens caused by *Clostridium perfringens*, as a way to reduce antimicrobial drug use in food animal production, and on the role of *Clostridium perfringens* in serious intestinal infections of animals. He was elected a Fellow of the Canadian Academy of Health Sciences in 2008.

2015 Mark Gearhart Memorial Graduate Student Award

CRWAD Presentation No. 320

Performance and carcass characteristics of commercial feedlot cattle from a study of vaccine and direct-fed microbial effects on *Escherichia coli* O157:H7 fecal shedding

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The objective of this study was to quantify cattle performance and carcass characteristics associated with administration of a siderophore receptor and porin proteins–based vaccine (VAC) and a direct-fed microbial (DFM), which were originally evaluated for their impact on *Escherichia coli* O157:H7 fecal shedding in a commercial feedlot population. Cattle (n = 17,148) were randomly allocated into 40 pens grouped by allocation dates into 10 complete blocks; pens within block were randomly allocated to control, VAC, DFM, or VAC + DFM treatment groups
in a 2 × 2 factorial design. The DFM (Bovamine) was fed daily at the labeled dose of 10^6 cfu/animal of *Lactobacillus acidophilus* for the duration of the intervention period (mean = 86.6 d). The VAC cattle were vaccinated on Days 0 and 21 whereas unvaccinated cattle were not given a placebo or rehandled on Day 21. Data were analyzed using general and generalized linear mixed models that accounted for the study design. Main effects of DFM and VAC are reported as there were no significant treatment interactions for any of the outcomes evaluated. Vaccinated cattle had lower total weight gain ($P < 0.01$), ADG ($P = 0.03$), and cumulative DMI during the intervention period ($P < 0.01$) compared with unvaccinated cattle, whereas the DFM increased total weight gain ($P = 0.03$) and G:F ($P = 0.05$) during the intervention period. Daily DMI was decreased ($P < 0.01$) in vaccinated pens compared with unvaccinated pens during a 5-d
period immediately following revaccination. After the intervention period was completed, cattle were sorted following the standard operating procedure for the feedlot and all cattle were fed the DFM from that point until harvest. Each steer was individually identified through harvest. At harvest, vaccinated cattle had more total days on feed ($P < 0.01$) with a larger HCW ($P = 0.01$) than nonvaccinated cattle, whereas cattle not fed the DFM during the intervention period had a significantly larger HCW ($P < 0.01$) than those fed the DFM during the intervention period. We conclude that the use of these DFM and vaccine products have differential and independent effects on cattle performance and carcass characteristics in a commercial feedlot setting.

2015 CRWAD Session Keynote Speakers

Bacterial Pathogenesis - Distinguished Veterinary Microbiologist – Dr. John. F. Prescott
Department of Pathobiology, University of Guelph, Ontario, ON, Canada
Monday, December 7, 10:45 PM – Avenue Ballroom, 4th Floor
No. 331 – "Driving through the fog: Understanding type A *Clostridium perfringens* in enteric disease of animals."

Biosafety and Biosecurity – Dr. Jason Stull
Department of Veterinary Preventive Medicine, Ohio State University, Columbus, OH, USA
Monday, December 7, 3:00 PM – Northwestern/Ohio - 6th Floor

No. 322 – "Infectious disease transmission risks on livestock farms: biosecurity practices, dogs and education."

Companion Animal Epidemiology,Ecology and Management of Foodborne Agents, and Epidemiology and Animal Health Economics – Dr.Scott J. Wells
College of Veterinary Medicine, University of Minnesota, St Paul, MN, USA.
Tuesday, December 8, 8:00 AM – Salon A/B/C/D – 5th Floor
No. 332 – "Experiential learning: The Farm to Table Study Program as a case study."

Immunology – Distinguished Veterinary Immunologist – Dr. Philip Griebel
School of Public Health and VIDO-Intervac, University of Saskatchewan, Saskatoon, SK, Canada
Monday, December 7, 1:30 PM – Salons F/G/H – 5th Floor
No. 306 – "First encounters: Mucosal immune system development and the microbiome."

Pathobiology of Enteric and Foodborne Pathogens – Dr. Roy Curtiss, III
College of Veterinary Medicine, University of Flordia, Gainsville, FL, USA
Monday, December 7, 8:45 AM – Michigan/Michigan State
No. 321 – "Enhancing food safety for human consumers by eliminating food-borne enteric pathogens."

Respiratory Diseases – Dr. Mike Clawson
USDA/ARS/ US Meat Animal Research Center, Clay Center, NE, USA.
Monday, December 7, 3:45 PM, Indiana/Iowa – 6th Floor
No. 221 – "Development of a nucleotide polymorphism-based typing method for *Mannheimia haemolytica* and identification of a subtype that associates with bovine respiratory disease."

Vector-Borne and Parasitic Diseases – Dr. Roman Ganta
Center of Excellence for Vector-Borne Diseases, Department of Diagnostic Medicine/Pathobiology, College of Veterinary Medicine, Kansas City
Monday, December 7, 10:00 AM, Denver/Houston – 5th Floor
No. "Molecular approaches in understanding *Ehrlichia* pathogenesis, host-pathogen interactions and in developing vaccines."

Viral Pathogenesis – Dr. Chang Won Lee
Veterinary Preventive Medicine and Food Animal Health Research Program, The Ohio State University, Wooster, OH, USA.
Tuesday, December 8, 10:00AM, Los Angeles/Miami
No. 335 – "Current understanding on intercontinental HPAI: To vaccinate or not?"
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Time	Oral #	Section	Monday- By-The-Day Title
8:00:00 AM	1	Bacterial Pathogenesis	Re-evaluating the LD50 requirements in the codified potency testing of Leptospira in the United States
8:00:00 AM	27	Companion Animal Epidemiology	Burden and predictors of Staphylococcus spp. infections among dogs presented at a veterinary academic hospital in South Africa
8:00:00 AM	50	Epidemiology and Animal Health Economics	Trends in antimicrobial resistance patterns of common Salmonella serotypes isolated from bovine samples in Wisconsin from 2006-2015
8:00:00 AM	83	Immunology	T lymphocytes induced after infection with a single PRRSV strain recognize epitopes processed from highly diverse PRRSV strains
8:00:00 AM	155	Vector-Borne and Parasitic Diseases	chaffeensis induces pathogen-specific CD4 T cell immunity and protection from wildtype challenge in a canine host
8:15:00 AM	2	Bacterial Pathogenesis	Dynamic attachment of differentially expressed Actinobacillus suis adhesins to host extracellular matrix components
8:15:00 AM	28	Companion Animal Epidemiology	Saving Fido - Unearthing a novel topical antimicrobial for treatment of multidrug-resistant staphylococcal skin infections in companion animals
8:15:00 AM	51	Epidemiology and Animal Health Economics	Temporal and geo-spatial characterization of Salmonella enterica serotypes isolated in Wisconsin from 2006 to 2015
8:15:00 AM	84	Immunology	Measuring bovine γδ T cell function at the site of Mycobacterium bovis infection
8:15:00 AM	112	Pathobiology of Enteric and Foodborne Pathogens	Characterization and application of monoclonal antibodies against porcine epidemic diarrhea virus
8:15:00 AM	135	Respiratory Diseases	ORF5 sequencing indicated PRRS strain shifting in the field
8:15:00 AM	156	Vector-Borne and Parasitic Diseases	Development and validation of real-time PCR assay for canine Lyme disease
8:15:00 AM	167	Viral Pathogenesis	Severe Fever with Thrombocytopenia Syndrome virus noncoding regions of S, M and L segments regulate RNA synthesis
8:30:00 AM	3	Bacterial Pathogenesis	Mycobacterium avium subspecies paratuberculosis from fecal samples of cattle for genomic epidemiology applications
8:30:00 AM	29	Companion Animal Epidemiology	System for specific and sensitive point-of-need detection of feline leukemia virus RNA and proviral DNA
8:30:00 AM	52	Epidemiology and Animal Health Economics	Genotypic characterization of extended-spectrum cephalosporin resistant nontyphoidal Salmonella from the NAHMS Feedlot 2011 study
8:30:00 AM	85	Immunology	Characterization of recombinant PRRSV nsp1beta mutants in a nursery pig model
8:30:00 AM	113	Pathobiology of Enteric and Foodborne Pathogens	Pathogenicity and physicochemical properties of SalmonellaTyphimurium treated with natural phenolics from industry byproducts

Time	Oral #	Section	Monday- By-The-Day Title
8:30:00 AM	136	Respiratory Diseases	Detection of Actinobacillus pleuropneumoniae ApxIV toxin antibody in serum and oral fluid specimens from pigs inoculated with under experimental conditions.
8:30:00 AM	157	Vector-Borne and Parasitic Diseases	Comparison of an alternative diagnostic sampling technique for Tritrichomonas foetus in cattle
8:30:00 AM	168	Viral Pathogenesis	Characterization of the humoral immune responses to porcine epidemic diarrhea Virus (PEDV) infection in weaned pigs
8:45:00 AM	4	Bacterial Pathogenesis	Improved diagnostics of Mycobacterium tuberculosis complex infections in Minnesota white-tailed deer
8:45:00 AM	30	Companion Animal Epidemiology	Temporal trends of feline retroviral infections diagnosed at the Ontario Veterinary College (1999-2012)
8:45:00 AM	53	Epidemiology and Animal Health Economics	Extended-spectrum cephalosporin and fluoroquinolone-resistant enterobacteriaceae in human and veterinary hospital environments
8:45:00 AM	86	Immunology	Reduced antigen-specific antibody levels in cows naturally infected with bovine leukemia virus
8:45:00 AM	114	Pathobiology of Enteric and Foodborne Pathogens	Enhancing food safety for human consumers by eliminating food-borne enteric pathogens
8:45:00 AM	137	Respiratory Diseases	Genetic diversity of porcine reproductive and respiratory syndrome virus genes determined by metagenomic sequencing of clinical samples
8:45:00 AM	158	Vector-Borne and Parasitic Diseases	Development of a recombinant subunit vaccine for Rift Valley fever
8:45:00 AM	169	Viral Pathogenesis	Porcine reproductive and respiratory syndrome virus hijacks nanotubes for intercellular spread: an alternative pathway used for nidovirus transmission
9:00:00 AM	5	Bacterial Pathogenesis	High throughput screening to identify quorum sensing inhibitors to enhance the control of avian pathogenic e. coli
9:00:00 AM	31	Companion Animal Epidemiology	Antimicrobial resistance in Escherichia coli isolated from equine clinical diagnostic specimens
9:00:00 AM	54	Epidemiology and Animal Health Economics	Spatial clustering of cefotaxime and ciprofloxacin resistant E. coli among dairy cattle relative to the European starling night roosts
9:00:00 AM	87	Immunology	Identification of immunodominant B cell epitopes in the C. pecorum proteome
9:00:00 AM	138	Respiratory Diseases	Discovery and pathogenesis of porcine parainfluenza-1 in pigs in the United States
9:00:00 AM	159	Vector-Borne and Parasitic Diseases	Fish mucus; a physical barrier to pathogens
9:00:00 AM	170	Viral Pathogenesis	protein attenuate the innate immune suppression function of porcine reproductive and respiratory syndrome virus (PRRSV)

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9:15:00 AM	6	Bacterial Pathogenesis	High-throughput screening to identify novel anti-campylobacter compounds using a pre-selected enriched small molecules library
9:15:00 AM	32	Companion Animal Epidemiology	Utility of electronic medical record data for healthcare-associated infection detection with fever sequella
9:15:00 AM	55	Epidemiology and Animal Health Economics	A survey of case-control studies in veterinary science
9:15:00 AM	88	Immunology	A synthetic biodegradable microsphere vaccine of femtomole-dosed peptide antigens protects better against Chlamydia abortus than previous infection
9:15:00 AM	139	Respiratory Diseases	Evaluation of the use of non-pathogenic porcine circovirus type 1 as a vaccine delivery virus vector to express antgenic determinants of porcine reproductive and respiratory syndrome virus
9:15:00 AM	160	Vector-Borne and Parasitic Diseases	Development of a generic Ehrlichia FRET-qPCR and investigation of ehrlichioses in domestic ruminants on five Caribbean islands
9:15:00 AM	171	Viral Pathogenesis	Attenuation of porcine reproductive and respiratory syndrome virus (PRRSV) by inactivating the ribosomal frameshifting products nsp2TF and nsp2N: Implication for the rational design of vaccines
10:00:00 AM	7	Bacterial Pathogenesis	directly identifies the genetic basis for emergent virulence caused by a rapidly expanding clone of Campylobacter jejuni
10:00:00 AM	33	Companion Animal Epidemiology	GRADE approach as a tool for making evidence-based decisions in veterinary medicine
10:00:00 AM	56	Epidemiology and Animal Health Economics	Risk of a first and recurrent case of pathogen specific clinical mastitis
10:00:00 AM	89	Immunology	15-F2t-Isoprostane concentrations correlate with oxidant status in lactating dairy cattle with acute coliform mastitis.
10:00:00 AM	115	Pathobiology of Enteric and Foodborne Pathogens	Salmonella Pathogenicity Island 13 contributes to pathogenesis in streptomycin pre-treated mice but not in day-old chickens.
10:00:00 AM	140	Respiratory Diseases	Agreement among sampling methods used to identify viral and bacterial pathogens in dairy calves with bovine respiratory disease (BRD)
10:00:00 AM	161	Vector-Borne and Parasitic Diseases	Molecular approaches in understanding Ehrlichia pathogenesis, host-pathogen interactions and in developing vaccines
10:00:00 AM	172	Viral Pathogenesis	Effects of adenoviral delivered interferon-alpha on porcine reproductive and respiratory syndrome virus infection in swine
10:15:00 AM	8	Bacterial Pathogenesis	Identification and characterization of virulence factors of staphylococcus aureus isolates from cases of bovine mastitis
10:15:00 AM	34	Companion Animal Epidemiology	Frequency, benefits and risks surrounding animals in Ohio nursing home facilities

Time	Oral #	Section	Monday- By-The-Day Title
10:15:00 AM	57	Epidemiology and Animal Health Economics	Pregnancy loss attributable to mastitis in first lactation Holstein cows
10:15:00 AM	90	Immunology	Regulation of host immune gene expression by Torque Teno Sus Virus1 (TTSuV1) non-structural proteins
10:15:00 AM	116	Pathobiology of Enteric and Foodborne Pathogens	Salmonella pathogenicity island 13 contributes to the metabolic fitness of Salmonella Enteritidis through glucuronic acid and tyramine metabolism.
10:15:00 AM	141	Respiratory Diseases	Iodine secretion in airway surface fluid following a single oral bolus of sodium iodide in calves.
10:30:00 AM	9	Bacterial Pathogenesis	Genetic mechanisms of Salmonella enterica serovar Typhimurium for overcoming host stressors
10:30:00 AM	35	Companion Animal Epidemiology	Epidemiologic aspects of fecal Salmonella and Campylobacter shedding among dogs at seven animal shelters across Texas
10:30:00 AM	58	Epidemiology and Animal Health Economics	The value of pathogen information in treating clinical mastitis.
10:30:00 AM	91	Immunology	Nsp1 and a part of Nsp2 genes of a synthetic porcine reproductive and respiratory syndrome virus are responsible for the viral capacity to induce type I interferons
10:30:00 AM	117	Pathobiology of Enteric and Foodborne Pathogens	Quantification of coliforms and Escherichia coli on beef carcasses immediately before and after evisceration during slaughter.
10:30:00 AM	142	Respiratory Diseases	In vitro inactivation of bovine viral respiratory pathogens using an iodine-based antimicrobial system
10:30:00 AM	173	Viral Pathogenesis	Both CD4+ and CD8+ T cells effectively suppress PRRSV replication in monocyte-derived macrophages
10:45:00 AM	10	Bacterial Pathogenesis	Driving through the fog: Understanding type A Clostridium perfringens in enteric disease of animals
10:45:00 AM	36	Companion Animal Epidemiology	An observational study of the prevalence of heartworm disease in Mississippi shelter dogs and test efficacy
10:45:00 AM	59	Epidemiology and Animal Health Economics	The effect of calf gender and age of dam on the risk for calves to develop bovine respiratory disease prior to weaning
10:45:00 AM	92	Immunology	Nanoparticle based Vaccination strategy against Swine Influenza Virus
10:45:00 AM	118	Pathobiology of Enteric and Foodborne Pathogens	Quantification of microbial transfer from hides to carcasses in commercial beef slaughter operations
10:45:00 AM	143	Respiratory Diseases	Sodium iodide inactivates Rhodococcus Equi in vitro
10:45:00 AM	162	Vector-Borne and Parasitic Diseases	Sequence determinants spanning -35 motif and AT-rich spacer sequences impacting ehrlichia chaffeensis sigma 70-dependent promoter activity of two differentially expressed p28 outer membrane protein genes

Time	Oral #	Section	Monday- By-The-Day Title
10:45:00 AM	174	Viral Pathogenesis	Both CD4+ and CD8+ T-cells recognize porcine reproductive and respiratory syndrome virus epitopes and lyse infected macrophages in a biphasic mode
11:00:00 AM	37	Companion Animal Epidemiology	Progression of surgical efficiency, incision length and complication rate in senior veterinary students enrolled in a 2 week spay/neuter surgical elective
11:00:00 AM	60	Epidemiology and Animal Health Economics	An update and model assessment of a mixed treatment comparison meta-analysis of antibiotic treatment for bovine respiratory disease
11:00:00 AM	93	Immunology	The impact of abomasal infusion of linoleic acid or linolenic acid on plasma fatty acid and oxylipid biosynthesis following Streptococcus uberis exposure
11:00:00 AM	119	Pathobiology of Enteric and Foodborne Pathogens	Bayesian estimation of sensitivity and specificity of culture- and PCR-based methods for the detection of the six major non-O157 Escherichia coli serogroups in cattle feces
11:00:00 AM	144	Respiratory Diseases	Sodium iodide inactivates Manheimia hemolytica and Bibersteinia trehalosi in vitro.
11:00:00 AM	163	Vector-Borne and Parasitic Diseases	Cerebral nematodiasis in camelids: a retrospective study
11:00:00 AM	175	Viral Pathogenesis	dependent RNA polymerase of porcine reproductive and respiratory syndrome virus play important roles in viral Ribavirin sensitivity and quasispecies diversity
11:15:00 AM	38	Companion Animal Epidemiology	Peri-operative morbidity and risk factors associated with routine sterilization surgeries performed on a mobile surgical service
11:15:00 AM	61	Epidemiology and Animal Health Economics	An investigation of piglet iron status at weaning and subsequent post-weaning growth performance
11:15:00 AM	94	Immunology	17 in response to respiratory syncytial virus and Mannheimia haemolytica: implications for bovine respiratory disease
11:15:00 AM	120	Pathobiology of Enteric and Foodborne Pathogens	Associations between diet, gut microbiome, and health in red-shanked doucs (Pygathrix nemaeus): a model for captive primate health
11:15:00 AM	145	Respiratory Diseases	Zelnate™: a novel approach to BRD management in cattle
11:15:00 AM	164	Vector-Borne and Parasitic Diseases	Molecular detection of vector-borne agents in dogs from ten provinces of China
11:15:00 AM	176	Viral Pathogenesis	Management Practices Implemented following an outbreak of Porcine Reproductive and Respiratory Syndrome in commercial swine breeding herds in North America
1:00:00 PM	11	Bacterial Pathogenesis	Our specific objectives were to examine the role of NetB in pathogenesis and the true place of alpha toxin (CPA), if any, in disease development.
1:00:00 PM	95	Immunology	First encounters: Mucosal immune system development and the microbiome

Time	Oral #	Section	Monday- By-The-Day Title
1:15:00 PM	121	Pathobiology of Enteric and Foodborne Pathogens	Lactobacillus reuteri derived-histamine suppress interleukin-6 by inhibiting H1-receptor downstream signaling in germ-free mice
1:30:00 PM	19	Biosafety and Biosecurity	Characteristics of infection control practices at North American veterinary teaching hospitals
1:30:00 PM	39	Ecology and Management of Foodborne Agents	Prevalence and characterization of Salmonella isolated from feral pigs throughout Texas
1:30:00 PM	62	Epidemiology and Animal Health Economics	A transboundary, epidemiologic simulation model for the spread and control of classical swine fever among commercial swine in the United States and Canada.
1:30:00 PM	146	Respiratory Diseases	Evaluation of response to vaccination on the feedlot performance of weaned calves.
1:30:00 PM	165	Vector-Borne and Parasitic Diseases	Defining the long-term duration of parasitemia and antibody response in cattle infected with various strains and doses of babesia bovis and evaluating sero-diagnostic tools
1:30:00 PM	177	Viral Pathogenesis	Attenuation of US original porcine epidemic diarrhea virus strain PC22A via continuous cell culture passages
1:45:00 PM	12	Bacterial Pathogenesis	Clostridium difficile infection - A One Health problem.
1:45:00 PM	20	Biosafety and Biosecurity	Evaluating environmental sampling methods for detection of S. enterica in a large animal veterinary hospital
1:45:00 PM	40	Ecology and Management of Foodborne Agents	Dissemination of carbapenem-resistant Enterobacteriaceae from a municipal wastewater treatment plant
1:45:00 PM	63	Epidemiology and Animal Health Economics	Quantification of strategies to mitigate animal-welfare concerns due to movement restrictions during a classical swine fever (CSF) outbreak
1:45:00 PM	96	Immunology	Mucosal immunology: microbial interaction and cytokine production
1:45:00 PM	122	Pathobiology of Enteric and Foodborne Pathogens	Host defense peptide-inducing compounds as alternatives to antibiotics
1:45:00 PM	147	Respiratory Diseases	Evaluation of on-arrival vaccination and deworming on stocker cattle health and growth performance
1:45:00 PM	166	Vector-Borne and Parasitic Diseases	The epidemiological status of African swine fever in domestic swine herds in the Tavush Marz region, Republic of Armenia.
1:45:00 PM	178	Viral Pathogenesis	Comparison of porcine epidemic diarrhea virus (PEDV), transmissible gastroenteritis virus (TGEV) and porcine deltacoronavirus (PDCoV) for pathogenicity in weaned pigs
2:00:00 PM	21	Biosafety and Biosecurity	The importance of being clean: biosecurity measures in farm operations.

Time	Oral #	Section	Monday- By-The-Day Title
2:00:00 PM	41	Ecology and Management of Foodborne Agents	Effect of copper, zinc, and essential oil supplementation on antimicrobial resistance of fecal Escherichia coli in nursery piglets
2:00:00 PM	64	Epidemiology and Animal Health Economics	Risk ranking of irish salmon farms based on network metrics and biosecurity evaluation
2:00:00 PM	148	Respiratory Diseases	Comparison of the immune response to subcutaneous or intranasal modified-live virus booster vaccination in young beef calves that were primed with intranasal vaccine
2:00:00 PM	179	Viral Pathogenesis	Prevalence of multi-drug resistance in E. coli and enterococci organisms isolated from abattoir workers and broilers
2:15:00 PM	13	Bacterial Pathogenesis	Carvacrol reduces Clostridium difficile virulence without inducing gut dysbiosis
2:15:00 PM	22	Biosafety and Biosecurity	Simultaneous detection of swine respiratory pathogens using a multiplexed detection assay
2:15:00 PM	42	Ecology and Management of Foodborne Agents	Antimicrobial susceptibility of enteric Gram-negative facultative anaerobe bacilli in aerobic versus anaerobic conditions
2:15:00 PM	65	Epidemiology and Animal Health Economics	Modeling the transmission of Orf in order to identify the mechanisms causing persistence.
2:15:00 PM	97	Immunology	An essential role of igt for pathogen clearance and microbiome homeostasis at mucosal surfaces of fish
2:15:00 PM	123	Pathobiology of Enteric and Foodborne Pathogens	From single probiotics to complex commensal microbiota: effects on immunity, enteric infections and vaccines in gnotobiotic pigs.
2:15:00 PM	149	Respiratory Diseases	Immune responses, clinical and pathological outcomes in challenged calves immunized with a subunit vaccine for BRSV and H.somni
2:15:00 PM	180	Viral Pathogenesis	A computationally designed indirect ELISA for the detection of porcine epidemic diarrhea virus (PEDV) - specific antibodies
2:30:00 PM	23	Biosafety and Biosecurity	Rapid detection of foot-and-mouth disease virus using a field-deployable, reverse-transcription insulated-isothermal PCR assay
2:30:00 PM	66	Epidemiology and Animal Health Economics	Filling gaps in notification data: a model-based approach applied to travel related campylobacteriosis cases in New Zealand
2:30:00 PM	150	Respiratory Diseases	Histophilus somni increases expression of antiviral proteins in bovine respiratory epithelial cells.
2:30:00 PM	181	Viral Pathogenesis	Does systemic antibody play a role in the protection of piglets against PEDV?
3:00:00 PM	14	Bacterial Pathogenesis	Biofilms, wounds, and chronic infections
3:00:00 PM	24	Biosafety and Biosecurity	Infectious disease transmission risks on livestock farms: biosecurity practices, dogs and education

Time	Oral #	Section	Monday- By-The-Day Title
3:00:00 PM	43	Ecology and Management of Foodborne Agents	Comparing the resistome of poultry, swine, cattle and salmon production and nearby human waste water treatment plants
3:00:00 PM	67	Epidemiology and Animal Health Economics	Role of carriers in the transmission dynamics of bighorn sheep pneumonia
3:00:00 PM	98	Immunology	Inflammatory mediator expression in lung epithelial cells and α/β T cells: roles in immunopathogenesis associated with respiratory syncytial virus infection in calves
3:00:00 PM	124	Pathobiology of Enteric and Foodborne Pathogens	Microbial shifts in the swine distal gut caused by the antimicrobial growth promoter tylosin
3:00:00 PM	151	Respiratory Diseases	Inhibition of Pasteurella multocida biofilm formation by capsular polysaccharide, and interaction with Histophilus somni in a polymicrobial biofilm
3:00:00 PM	182	Viral Pathogenesis	Transduction of hematopoietic stem cells to stimulate RNA interference for treatment of feline infectious peritonitis
3:15:00 PM	44	Ecology and Management of Foodborne Agents	Use of shotgun metagenomic to evaluate the microbiome in cattle feces following tulathromycin metaphylaxis
3:15:00 PM	68	Epidemiology and Animal Health Economics	Developing sampling guidelines for oral fluid-based PRRSV surveillance
3:15:00 PM	152	Respiratory Diseases	Genetic engineering of cattle that produce leukocytes resistant to Mannheimia haemolytica leukotoxin
3:15:00 PM	183	Viral Pathogenesis	Development of a snatch farrowed-colostrum deprived piglet challenge model for porcine Rotavirus C
3:30:00 PM	45	Ecology and Management of Foodborne Agents	Characterizing variation in the microbial resistome between natural and conventional beef operations
3:30:00 PM	69	Epidemiology and Animal Health Economics	Detecting human brucellosis in rural Uganda: Comparison of a commercial lateral flow assay with microagglutination on sera from high-risk subjects
3:30:00 PM	99	Immunology	PIV-3 blocks antiviral mediators downstream of the IFN-λR by modulating Stat1 phosphorylation
3:30:00 PM	125	Pathobiology of Enteric and Foodborne Pathogens	The hunt for alternatives to antibiotics in poultry: a systematic, microbial community-based approach
3:30:00 PM	153	Respiratory Diseases	Mannheimia haemolytica leukotoxin is cytotoxic even in the absence of acylation.
3:30:00 PM	184	Viral Pathogenesis	Evaluation of clinical and immune responses following infection of horses with EHV-1 wild type and different EHV-1 mutants
3:45:00 PM	15	Bacterial Pathogenesis	role during systemic and central nervous system infections by Streptococcus suis strains from different backgrounds
3:45:00 PM	25	Biosafety and Biosecurity	Development of an animal model for Schmallenberg virus

Time	Oral #	Section	Monday- By-The-Day Title
3:45:00 PM	46	Ecology and Management of Foodborne Agents	Repeated oral immunization with Shiga toxin negative Escherichia coli O157:H7 transiently reduces carriage of wild-type EHEC O157 by cattle following oral challenge
3:45:00 PM	70	Epidemiology and Animal Health Economics	Comparative study of diagnostic tests for Tuberculosis in cattle
3:45:00 PM	100	Immunology	From Swine Dysentery to Inflammatory Bowel diseases: Role of the Resident Microbiota in Tuning the Host Response.
3:45:00 PM	154	Respiratory Diseases	typing method for Mannheimia haemolytica and identification of a subtype that associates with bovine respiratory disease
3:45:00 PM	185	Viral Pathogenesis	Development of a real-time PCR assay for the detection and quantification of equine herpesvirus 5
4:00:00 PM	16	Bacterial Pathogenesis	Interactions between Streptococcus suis and Haemophilus parasuis
4:00:00 PM	26	Biosafety and Biosecurity	Polymorphism analysis of prion protein gene in eleven pakistani goat breeds
4:00:00 PM	47	Ecology and Management of Foodborne Agents	Risk profile and quantitative exposure assessment of hepatitis E virus from pigs or pork in Canada
4:00:00 PM	71	Epidemiology and Animal Health Economics	Assay with other serological tests and Polymerase Chain Reaction for the diagnosis of Brucellosis in livestock
4:00:00 PM	126	Pathobiology of Enteric and Foodborne Pathogens	Commensal gut bacteria as new generation probiotics to improve gut health
4:00:00 PM	186	Viral Pathogenesis	Sites of equine arteritis virus persistence in the stallion's reproductive tract and characterization of the local inflammatory response to the virus
4:15:00 PM	17	Bacterial Pathogenesis	Biofilm formation by Mannheimia haemolytica on bovine bronchial epithelial cells.
4:15:00 PM	72	Epidemiology and Animal Health Economics	Detection of Dichelobacter nodosus and Fusobacterium necrophorum from footrot of sheep and goats in Andhra Pradesh: Southern India.
4:15:00 PM	101	Immunology	PEDV shedding patterns and antibody kinetics in commercial growing pigs
4:15:00 PM	187	Viral Pathogenesis	Equine arteritis virus uses equine CXCL16 (EqCXCL16) as a cell entry receptor
4:30:00 PM	18	Bacterial Pathogenesis	Mannheimia haemolytica biofilm cells induce neutrophil extracellular trap (NET) formation in vitro
4:30:00 PM	127	Pathobiology of Enteric and Foodborne Pathogens	The swine intestinal microbiota: localized responses to in-feed antibiotics

Time	Oral #	Section	Tuesday - By-The Day
8:00:00 AM	48	Ecology and Management of Foodborne Agents	Experiential learning: The Farm to Table Study Program as a case study
8:00:00 AM	73	Epidemiology and Animal Health Economics	Experiential learning: The Farm to Table Study Program as a case study
8:00:00 AM	102	Immunology	Cellular response following Digital Dermatitis infection
8:15:00 AM	103	Immunology	Montanide™ adjuvant technologies for influenza vaccines.
8:15:00 AM	128	Pathobiology of Enteric and Foodborne Pathogens	A highly-sensitive, field-deployable molecular assay for rapid detection of porcine epidemic diarrhea virus
8:15:00 AM	188	Viral Pathogenesis	Application of a broad-spectrum microbial detection array for the analysis of pig pathogens
8:30:00 AM	104	Immunology	Expression of interferon-beta (IFN-β) by dendritic cells activated with Streptococcus suis
8:30:00 AM	129	Pathobiology of Enteric and Foodborne Pathogens	Bayesian estimation of sensitivity and specificity of culture- and PCR-based methods for the detection of Escherichia coli O157 in cattle feces
8:30:00 AM	189	Viral Pathogenesis	Detection of antibody responses to the porcine circovirus strain 2 (PCV2) replicase protein.
8:45:00 AM	49	Ecology and Management of Foodborne Agents	Performance and carcass characteristics of commercial feedlot cattle from a study of vaccine and direct-fed microbial effects on Escherichia coli O157:H7 fecal shedding
8:45:00 AM	74	Epidemiology and Animal Health Economics	Performance and carcass characteristics of commercial feedlot cattle from a study of vaccine and direct-fed microbial effects on Escherichia coli O157:H7 fecal shedding
8:45:00 AM	105	Immunology	Intramammary 25-hydroxyvitamin D3 treatment increases vitamin D pathway activity but not acute host-defense responses to endotoxin-induced mastitis.
8:45:00 AM	130	Pathobiology of Enteric and Foodborne Pathogens	Optimizing & standardizing anti-STa antibody titration assay by using ovalbumin-STa fusion protein as ELISA coating antigen
8:45:00 AM	190	Viral Pathogenesis	Discovery of a novel putative atypical porcine pestivirus in pigs in the United States
9:00:00 AM	75	Epidemiology and Animal Health Economics	Understanding PEDV transmission by live haul transport at swine lairage facilities
9:00:00 AM	106	Immunology	A novel vector platform for vaccine delivery in domestic animal species
9:00:00 AM	131	Pathobiology of Enteric and Foodborne Pathogens	A multiepitope fusion antigen of fimbrial adhesin tips of enterotoxigenic Escherichia coli (ETEC) indices broadly protective anti-adhesin antibodies
9:00:00 AM	191	Viral Pathogenesis	Identification of novel Senecavirus A from pigs with vesicular disease in the US

Time	Oral #	Section	Tuesday - By-The Day
9:15:00 AM	76	Epidemiology and Animal Health Economics	Developing sampling guidelines for PEDV surveillance
9:15:00 AM	107	Immunology	Porcine reproductive and respiratory syndrome virus non-structural protein Nsp2TF down-modulates Swine Leukocyte Antigen class I (SLA class I) expression
9:15:00 AM	132	Pathobiology of Enteric and Foodborne Pathogens	Adjuvanticity of double mutant heat-labile toxin (dmLT, LTR192G/L211A) of enterotoxigenic Escherichia coli (ETEC) in mouse parenteral immunizations with a toxoid fusion antigen
9:15:00 AM	192	Viral Pathogenesis	Protective properties of live and inactivated vaccine based on rescued recombinant influenza A virus against highly pathogenic H5N1 strain in chickens.
10:00:00 AM	77	Epidemiology and Animal Health Economics	Mental health and wellness in veterinarian and agricultural producers in Ontario, Canada
10:00:00 AM	108	Immunology	Endonuclease G participates in caspase-independent apoptosis induced by Mycobacterium bovis in bovine macrophages.
10:00:00 AM	133	Pathobiology of Enteric and Foodborne Pathogens	Effect of ascorbic acid on survival and bacterial contents in the gut contents of oreochromis niloticus
10:00:00 AM	193	Viral Pathogenesis	Current understanding on intercontinental HPAI: To vaccinate or not?
10:15:00 AM	78	Epidemiology and Animal Health Economics	Comparison of Johne's disease prevalence on organic and conventional dairy farms in Pennsylvania
10:15:00 AM	109	Immunology	Pathogenesis comparison of the U.S. PEDV prototype and S-INDEL-variant strains in weaned pigs and examination of the cross-protective immunity of two virus strains
10:15:00 AM	134	Pathobiology of Enteric and Foodborne Pathogens	Identification of potential probiotic species for growth promotion in turkey flocks.
10:30:00 AM	79	Epidemiology and Animal Health Economics	Meta-analysis of the effects of laidlomycin propionate or monensin sodium on performance, health, and carcass outcomes in finishing steers in North America
10:30:00 AM	110	Immunology	Genome wide association study identifies loci associated with somatic cell count phenotypes following experimental challenge with Streptococcus uberis
10:45:00 AM	80	Epidemiology and Animal Health Economics	Cluster analysis of Campylobacter isolates obtained from beef cattle, dairy cattle, swine, and mammalian wildlife on Southern Ontario farms
10:45:00 AM	111	Immunology	Bordetella bronchiseptica colonization has minimal impact on live-attenuated influenza virus vaccine cross-protective efficacy in pigs.
10:45:00 AM	194	Viral Pathogenesis	Mouse model for the Rift Valley Fever virus MP12 strain infection
11:00:00 AM	81	Epidemiology and Animal Health Economics	Isolation and characterization of Salmonella spp. from captive wild animals

Time	Oral #	Section	Tuesday - By-The Day
11:00:00 AM	195	Viral Pathogenesis	Identification and serotyping of Foot and Mouth Disease virus prevalent in Savar upazila of Bangladesh using one-step RT-PCR and multiplex RT-PCR
11:15:00 AM	82	Epidemiology and Animal Health Economics	Following Salmonella Heidelberg through a poultry integrator.

POSTER PROGRAM

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Bacterial Pathogenesis/Gireesh Rajashekara

No.	Title	Author
001p	The role of Fur in Iron Regulation during Mycobacterium avium subsp. paratuberculosis infection - Johne's disease	**F.M. Shoyama**, E. Lamont, S. Sreevatsan;Veterinary Population Medicine, University of Minnesota, Saint Paul, MN, USA.
002p	Assessment of effect of bacteriophage in post-weaning pigs challenged with enterotoxigenic Escherichia coli K88 and K99	**J. Han**, College of Veterinary Medicine, Kangwon National University, Chuncheon, Korea, Republic of.
003p	Differential gene expression of mastitis-causing Escherichia coli due to swarming, swimming, and planktonic growth conditions	**J.D. Lippolis**[1], B.W. Brunelle[2], T.A. Reinhardt[1], R.E. Sacco[1], T.C. Thacker[3];1Ruminant Disease and Immunology, National Animal Disease Center / ARS / USDA, Ames, IA, USA, 2Food Safety and Enteric Pathogens, National Animal Disease Center / ARS / USDA, Ames, IA, USA, 3Bacterial Diseases of Livestock, National Animal Disease Center / ARS / USDA, Ames, IA, USA.
004p	Assessment of serological effect of SUISENG® against preweaning diarrhea under field condition in South Korea	**J. Han**, College of Veterinary Medicine, Kangwon National University, Chuncheon, Korea, Republic of.
005p	Effects of supplementation of lipid-encapsulated zinc oxide in weaned piglets with colibacillosis challenged with enterotoxigenic E. coli K88	**J. Han**, College of Veterinary Medicine, Kangwon National University, Chuncheon, Korea, Republic of.
006p	Evaluation of SUISENG® efficacy on Korean field trial	**J. Han**, College of Veterinary Medicine, Kangwon National University, Chuncheon, Korea, Republic of.
007p	Vaccination to prevent brucellosis in elk (cervus canadensis).	**S. Olsen**, National Animal Disease Center, Ames, IA, USA.
008p	Targeting methicillin resistant Staphylococcus pseudintermedius (MRSP) with novel antimicrobial peptides	**M.F. Mohamed**[1], G.K. Hammac[1], L. Guptill[2], M. Seleem[1];1Comparative pathobiology, purdue university, west lafayette, IN, USA, 2Veterinary Clinical Sciences, purdue university, west lafayette, IN, USA.
009p	Inter- and intra-serotype differences in the uptake, survival and modulation of nitric oxide production in avian macrophages (HD-11) by the most prevalent poultry-associated Salmonella seroTypes	W.A. Elnyaad, N.C. Paul, **D.H. Shah**,Department of Veterinary Microbiology and Pathology, Washington State University, Pullman, WA, USA.
010p	Preliminary sequence comparisons of six Leptospira strains	E. Bajak[1], **S. Rajeev**[2];1Biomedical Sciences, Ross University School of Veterinary Medicine, Basseterre, Saint Kitts and Nevis, 2Biomedical Sciences, Ross University School of Veterinary Medicine, Baasseterre, Saint Kitts and Nevis.
011p	Risk analysis of Q-fever entry in the territory of Ukraine	**L. Marushchak**, Molecular genetic investigation, State Institute of Laboratory Diagnostics, Kyiv, Ukraine.
012p	Comparative genomic analysis of two netF-positive Clostridium perfringens isolates associated with foal necrotizing enteritis and canine haemorrhagic gastroenteritis.	**I. Mehdizadeh Gohari**, A. Kropinski, P. Boerlin, J.F. Prescott, Pathobiology, University of Guelph, Guelph, ON, Canada.

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BioSafety and BioSecurity/Brandy Burgess

No.	Title	Author
013p	Status of laboratory biosafety and biosecurity in veterinary research facilities in Nigeria	**I.A. Odetokun**[1], A.T. Jagun-Jubril[2], B.A. Onoja[3], Y.S. Wungak[4], J.C. Chen[5], C.G. Campos[6];1Department of Veterinary Public Health and Preventive Medicine, University of Ilorin, Ilorin, Nigeria, 2Department of Veterinary Pathology, University of Ibadan, Ibadan, Nigeria, 3Department of Virology, University of Ibadan, Ibadan, Nigeria, 4Viral Research Division, National Veterinary Research Institute, Vom, Nigeria, 5Department of Food Science, University of British Columbia, Vancouver, BC, Canada, 6National Biosafety and Biocontainment Training Program (NBBTP), National Institutes of Health, DOHS, Bethesda, MD, USA.
014p	Development of real-time PCR methods for rapid detection and quantification of two marine harmful algal bloom microalgae: Karenia mikimotoi and Prorocentrum donghaiense	**J. Yuan**[1], T. Mi[2], Y. Zhen[2], Z. Yu[3], S. Ensley[1], K.-J. Yoon[1];1Department of VDPAM, College of Vet Med, Iowa State University, Ames, IA, USA, 2Department of Environmental Sciences, College of Environmental Science and Engineering, Ocean University of China, Qingdao, China, 3Department of Marine Chemistry, College of Chemistry and Chemical Engineering, Ocean University of China, Qingdao, China.
015p	Bluetongue, Akabane and Schmallenberg virus surveillance in Kazakhstan	**T. Karibayev**[1], I. Sytnik[1], S. Tyulegenov[1], D. Sembaeva[1], A. Tashkenbayev[1], S. Yerimbetov[1], R. Kozhakhmetov[1], Z. Aitzhanov[1], A. Tegzhanov[1], A. Akhmetova[1], K. Patel[2], S. Francesconi[2];1National Reference Veterinary Center, Astana, Kazakhstan, 2Naval Medical Research Center, Frederick, MD, USA.

Companion Animals Epidemiology/ Audrey Ruple-Czerniak and Laura Hungerford

016p	Spatial and temporal distribution of brucella isolates recovered from slaughtered cows in Egypt during the years of 2010-2015	**H.H. Abdalaal**, Sr. ; Veterinary Medicine, Beni Suef university, Beni Suef, Egypt.
017p	Spatial and temporal distribution of brucella isolates recovered from slaughtered cows in egypt during the years of 2010-2015.h.i.hosein1, a.menshawy2, sherin r. rouby3 and ayman mahrous4. 1,2,3 dept. of veterinary medicine, college of veterinary medicine, beni suef university, egypt. 4 govs, giza, egypt.	**H.I.H. Abdalaal**, Sr. , A.M. Menshawy, S.R. Rouby; Veterinary Medicine, Beni Suef university, Beni Suef, Egypt.

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Companion Animals Epidemiology/ Audrey Ruple-Czerniak and Laura Hungerford

No.	Title	Author
018p	recovered from slaughtered cows in egypt during the years of 2010-2015.h.i.hosein1*, a.menshawy2, sherin r. rouby3 and ayman mahrous4. 1,2,3 dept. of veterinary medicine, college of veterinary medicine,	**H.I.H. Abdalaal**, Sr. ; Veterinary Medicine, Beni Suef university, Beni Suef, Egypt.
019p	Bacteriological and molecular characterization of Brucella melitensis in Dromedary camels in Egypt	**H.I. Hosein**, S.R. Rouby; Veterinary Medicine, College of Veterinary Medicine, cairo, Egypt.
020p	Evidence of Mycobacterium bovis in a dog population that cohabits with cattle	**I.N. Jiménez Vázquez**[1], E. Morales Salinas[2], R. Acosta Salinas[3], O. Soberanis Ramos[1], A. Benítez Guzmán[4], E. Alfonseca Silva[4], R.E. Méndez Aguilar[5], J.A. Gutiérrez Pabello[4];1Medicina Preventiva y Salud Pública, Universidad Nacional Autónoma de México, México D.F, Mexico, 2Patología, Universidad
021p	Monitoring of circulation of H1, H2, H3 “human” influenza viruses among wild birds in different ecological groups of Ukraine in 2006-2012	**D. Muzyka**[1], B. Stegniy[1], M. Pantin-Jackwood[2], A. Stegniy[1];1Avian Diseases Epizootology, National Scientific Center – Institute of Experimental and Clinical Veterinary Medicine, Kharkiv, U, Kharkiv, Ukraine, 2Southeast Poultry Research Laboratory, Athens, GA, USA.

Epidemiology and Animal Economics/Ashley Hill

No.	Title	Author
022p	Environmental surveillance for bacterial extended spectrumβbeta-lactamase resistance genes at a municipal wastewater treatment plant	**C.A. King**[1], D.F. Mollenkopf[1], D.A. Mathys[1], D.M. Stuever[1], J.B. Daniels[2], T.E. Wittum[1];1Veterinary Preventive Medicine, The Ohio State University, Columbus, OH, USA, 2Veterinary Clinical Sciences, The Ohio State University, Columbus, OH, USA.
023p	Antimicrobial susceptibility profiles of MRSA ST5 isolates from swine production settings and clinical isolates from humans with no swine contact	**S.J. Hau**[1], T. Frana[1], P.R. Davies[2], J. Sun[3], T.L. Nicholson[4];1Department of Veterinary Diagnostic and Production Animal Medicine, College of Veterinary Medicine, Iowa State University, Ames, IA, USA, 2Department of Veterinary Population Medicine, College of Veterinary Medicine, University of Minnestoa, St. Paul, MN, USA, 3Department of Veterinary Populatino Medicine, College of Veterinary Medicine, University of Minnesota, St. Paul, MN, USA, 4National Animal Disease Center, Agricultural Research Service, USDA, Ames, IA, USA.
024p	Dissemination of antimicrobial resistant enteric bacteria in a zoo environment	**S. Feicht;** Veterinary Preventive Medicine, Ohio State University College of Veterinary Medicine, Columbus, OH, USA.
025p	Longitudinal monitoring of small layer flocks with less than 3,000 hens for Salmonella Enteritidis	**T. Denagamage;** Veterinary and Biomedical Sciences, The Pennsylvania State University, University Park, PA, USA.
026p	Investigation of biofilm producing ability in Staphylococcus spp. isolated from buffalo milk and milking environment using phenotypic and genotypic assays	**C.C. de Almeida**[1], L.J.L. Pizauro[1], J.I. MacInnes[2], G.A. Soltes[2], O.D. Rossi Junior[1], F. de Ávila[1], J. Pizauro-Junior[1];1University of Sao Paulo State - UNESP, Jaboticabal, Brazil, 2Pathobilogy, University Of Guelph, Guelph, ON, Canada.

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Epidemiology and Animal Economics/Ashley Hill

No.	Title	Author
027p	Characterization of Staphylococcus spp. isolated from buffalo milk by Matrix Associated Laser Desorption-Ionization - Time of Flight Mass spectrometry (MALDI_TOF MS)	**L.J.L. Pizauro**[1], C.C. de Almeida[2], J.I. MacInnes[2], D. Slavic[2], G.A. Soltes[2], O.D. Rossi-Junior[3], F.A. de Ávila[3], L.F. Zafalon[4];1Sao Paulo State University - UNESP, Jaboticabal, Brazil, 2University Of Guelph, Guelph, ON, Canada, 3University of Sao Paulo State - UNESP, Jaboticabal, Brazil, 4Empresa Brasileira de Pesquisa Agropecuária EMBRAPA, São Carlos, Brazil.
028p	The effect of weaning stress, sex and temperament on fecal microbiota in Brahman calves.	**E.V. Gart**[1], T.H. Welsh, Jr[2], R.D. Randel[3], J.S. Suchodolski[1], J. Kintzinger[1], S.D. Lawhon[1];Veterinary Medicine, Texas A&M University, College Station, TX, USA, 2Animal Science, Texas A&M University, College Station, TX, USA, 3Texas A&M AgriLife Research, Overton, TX, USA
029p	Investigation of Porcine Deltacoronavirus in Indiana swine population.	H.-Y. Cha[1], C. Schnur[1], **R.M. Pogranichniy**[2];1Comperative Pathobiology, Purdue University, W. Lafayette, IN, USA, 2Comparative Pathobiology, Veterinary college., Purdue University, W. Lafayette, IN, USA.
030p	Prevalence of Brucellosis in dairy cattle in South Korea with special emphasis on epidemiology, risk factors and control opportunities	**J. Lee**, M. Her, S. Sung, J. Kim, S. Kang, S. Kim, H. Lee; Animal and Plant Health Research, Animal and Plant Quarantine Agency, Anyang, Korea, Republic of.
031p	Evolutionary analysis of highly pathogenic avian influenza H5N2 outbreak in Minnesota and neighboring states	**N. Nonthabenjawan**, C. Cardona, S. Sreevatsan; Department of Veterinary Population Medicine, College of Veterinary Medicine, University of Minnesota, Saint Paul, MN, USA.
032p	Study of population awareness, attitude, and behavior in relation with rabies in animals in Georgia	**L. Ninidze**[1], T. Chkuaseli[2];1National Food Agency (NFA) of the Ministry of Agriculture (MoA), Tbilisis, Georgia, 2Laboratory of the Ministry of Agriculture (LMA), Tbilisis, Georgia.
033p	Active surveillance of African swine fever in commercial swine herds in the Republic of Georgia in 2014.	**N. Vepkhvadze**[1], M. Kokhreidze[1], K. Goginashvili[1], T. Tigilauri[1], E. Mamisashvili[1], L. Gelashvili[1], T. Abramishvili[1], M. Donduasvili[1], O. Parkadze[2], L. Ninidze[2], N. Kartskhia[2], R. Weller[3], G. Risatti[4];1Laboratory of the Ministry of Agriculture (LMA), Tbilisis, Georgia, 2National Food Agency (NFA), Tbilisis, Georgia, 3Fundamental Computational and Sciences Division, Pacific Northwest National Laboratory, Richland, WA, USA, 4Department of Pathobiology and Veterinary Science, CAHNR, University of Connecticut, Storrs, CT, USA.
034p	with the strain stern bacillus anthracis. dr.t. bajrovic1, a.celes2. 1university of veterinary medicine, department of microbiology with immunology and infectious diseases with epizootiology, sarajevo, bosnia-	**T. Bajrovic**[1], A. Celes[2];1Dept of Microbiology with Immunology, Veterinary University of Sarajevo, Sarajevo, Bosnia and Herzegovina, 2Veterinary Clinic, Knezevo, Bosnia and Herzegovina
035p	Pilot extension program to control bovine leukemia virus in michigan dairy farms	**V.J. Ruggiero,** P.T. Durst, D.L. Grooms, R.J. Erskine, B. Norby, R. LaDronka, L.M. Sordillo, P.C. Bartlett; Michigan State University, East Lansing, MI, USA.
036p	A national survey of bovine leukemia virus: preliminary descriptive epidemiology	Bartlett[2];1Comparative Medicine and Integrative Biology, Michigan State Universtiy, College of Veterinary Medicine, East Lansing, MI, USA, 2Large Animal Clinical Sciences, Michigan State Universtiy, College of Veterinary Medicine, East Lansing, MI, USA, 3Antel BioSystems, Lansing, MI, USA.

Poster Session I - Sunday 6:30 - 8:00 PM - Grandballroom Salon III - 7th Floor Poster Assembly begins at 4 PM Sunday. Please remove your poster by 10:00 AM Monday. Poster Presenters must be with their competiton entry posters for possible judge interviews. Name badge are required.

Epidemiology and Animal Economics/Ashley Hill

No.	Title	Author
037p	Field trial methodology to reduce transmission of bovine leukemia virus in Midwestern US dairy herds	**V.J. Ruggiero,** P.C. Bartlett, R.J. Erskine, B. Norby, L.M. Sordillo; Michigan State University, East Lansing, MI, USA.

Pathobiology of Enteric and Foodborne Pathogens/Weiping Zhang

038p	Host specificity of Lactobacillus johnsonii isolated from commercial turkeys	**K.A. Case**[1], B.P. Youmans[1], S. Noll[2], J. Scalze[1], B. Lindquist[3], J. Danzeisen[1], T. Johnson[1];1Veterinary Bioscience, University of Minnesota, Falcon Heights, MN, USA, 2Animal Sciences, University of Minnesota, Falcon Heights, MN, USA, 3Lesaffre Feed Additives, Humboldt, IA, USA.
039p	Analysis of a comprehensive Himar1 transposon library of Mycobacterium avium subsp. paratuberculosis.	Grohn[3], R.G. Barletta[1];1School of veterinary Medicine and Bio-medical Sciences, University of Nebraska, Lincoln, NE, USA, 2National Animal Disease Center, Ames, IA, USA, 3Population Medicine and Diagnostic Sciences, Cornell University, Ithaca, NY, USA.
040p	Targeting intracellular pathogenic bacteria with a kanamycin antibiotic peptide conjugate	**M.F. Mohamed**[1], A. Brezden[2], J. Chmielewski[2], M. Seleem[1];1Comparative pathobiology, purdue university, west lafayette, IN, USA, 2Chemistry, purdue university, west lafayette, IN, USA.
041p	Immune response and protective efficacy of intranasal vaccination of subunit vaccines for Campylobacter control in broilers	X. Liu, X. Zeng, B. Gillespie, **J. Lin**; Dept. of Animal Science, University of Tennessee, Knoxville, TN, USA.
042p	Lectin binding profiles of primary and immortalized bovine intestinal epithelial cell cultures (BIECs) and infectivity of primary BIECs to different enteric viral pathogens	P. Katwal[1], T. Milton[1], L. Elmore[1], F. Okda[2], L. Braun[2], C. Chase[2], D. Diel[2], E. Nelson[2], **R. Kaushik**[3];1Dept of Biology and Microbiology, South Dakota State University, Brookings, SD, USA, 2Dept of Veterinary and Biomedical Sciences, South Dakota State University, Brookings, SD, USA, 3Dept. of Biology and Microbiology, and Dept of Veterinary and Biomedical Sciences, South Dakota State University, Brookings, SD, USA.

Poster Session II - Monday 5:00 - 6:30 PM - Grandballroom Salon III - 7th Floor Poster Assembly begins at 12:00 PM Monday. Please remove your poster by 6:30 PM Monday. Poster Presenters must be with their competiton entry posters for possible judge interviews.
Name badge are required.

Ecology and Management of Foodborne Agents/Tom Wittum

No.	Title	Author
43p	Knowledge, attitude and practices associated with Bovine Brucellosis risk and transmission among occupationally exposed individuals in Ibadan, Nigeria.	P.I. Alabi[1], T.O. Isola[1], **I.A. Odetokun**[2], R.C. Okocha[1];1Department of Veterinary Public Health and Preventive Medicine, University of Ibadan, Ibadan, Nigeria, 2Department of Veterinary Public Health and Preventive Medicine, University of Ilorin, Ilorin, Nigeria.
44p	Assessment of antimicrobial activity of chlorine against most prevalent poultry-associated Salmonella serotypes in a chicken-meat-based model	N.C. Paul[1], T.S. Sullivan[2], **D.H. Shah**[1];1Department of Veterinary Microbiology and Pathology, Washington State University, Pullman, WA, USA, 2Department of Crop and Soil Sciences, Washington State University, Pullman, WA, USA.
45p	Population dynamics and antimicrobial resistome of the most prevalent poultry-associated Salmonella serotypes isolated from the US poultry	**D.H. Shah**[1], N.C. Paul[1], W.C. Sischo[2], R. Crespo[1], J. Guard[3];1Department of Veterinary Microbiology and Pathology, Washington State University, Pullman, WA, USA, 2Department of Veterinary Clinical Sciences, Washington State University, Pullman, WA, USA, 3Egg Quality and Safety Research Unit, United States Department of Agriculture, Atlanta, GA, USA.
46p	Antimicrobial resistance and molecular characterization of Salmonella Kentucky isolates from human and poultry	C.K. Sakamoto[1], N.C. Paul[1], W.C. Sischo[2], **D.H. Shah**[1];1Department of Veterinary Microbiology and Pathology, Washington State University, Pullman, WA, USA, 2Department of Veterinary MClinical Sciences, Washington State University, Pullman, WA, USA.
47p	The role of species richness in infectious disease studies: A preliminary inquiry based upon Coxiella burnetii	**A.E. Bauer**, A.J. Johnson, H.-Y. Weng; Comparative Pathobiology, Purdue University, Lafayette, IN, USA.
48p	Quantification of six major non-O157 Escherichia coli serogroups in cattle hide samples by spiral plating and multiplex quantitative PCR methods	**P. Belagola Shridhar**, L.W. Noll, C.A. Cull, A. Mckieaman, X. Shi, N. Cernicchiaro, M.W. Sanderson, D.G. Renter, J. Bai, T.G. Nagaraja; College Of Veterinary Medicine, Manhattan, KS, USA.
49p	Comparison of the microbiological quality of fresh produce from seasonal farmer's markets and retail grocery stores in Ohio.	**D.I. Korec,** D.A. Mathys, D.F. Mollenkopf, T.E. Wittum; Department of Veterinary Preventive Medicine, College of Veterinary Medicine, Ohio State University, Columbus, OH, USA.
50p	Validation of single and pooled manure drag swabs for the detection of Salmonella ser. Enteritidis in commercial poultry houses	H. Kinde[1], H.A. Goodluck[1], M. Pitesky[2], T.D. Friend[3], J.A. Campbell[4], **A.E. Hill**[5];1California Animal Hlth & Food Safety Lab, University of California-Davis, San Bernardino, CA, USA, 2Population Health and Reproduction, University of California-Davis, Davis, CA, USA, 3MCM Poultry Farm, Colton, CA, USA, 4Animal Health Branch, California Dept of Food and Agriculture, Ontario, CA, USA, 5California Animal Hlth & Food Safety Lab, University of California-Davis, Davis, CA, USA.
51p	A short review of policies and indications of antimicrobial drugs for food animals in the U.S.	**Z. DeMars**, V. Volkova; Department of Diagnostic Medicine/Pathobiology, College of Veterinary Medicine, Kansas State University, Manhattan, KS, USA.
52p	Seasonal Escherichia coli O157:H7 infection of cattle using standardized doses demonstrate factors extrinsic to the animal drive increased summertime colonization	H. Sheng[1], **S. Shringi**[2], K.N.K. Baker[2], S.A. Minnich[1], C.J. Hovde[1], T.E. Besser[2];1Bi-state School of Food Science, University of Idaho, Moscow, ID, USA, 2Washington State Univesity, Pullman, WA, USA.
89p	Methicillin-resistant Staphylococcus aureus in beef products in Sulaimaniyah City-Iraq	H.A. Qadir; Veterinary Directorate, Kurdistan Ministry of Agriculture and Water Reseurces, KRG, Iraq.

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Name badge are required.

Immunology/Renukaradhya Gourapura and Radhey Kaushik

No.	Title	Author
53p	Vitamin D3 increases nitric oxide production in Mycobacterium bovis-infected bovine macrophages.	Á. García Barragán, E. Alfonseca Silva, J. Gutiérrez Pabello; Laboratorio de Investigación en Tuberculosis Bovina, Departamento de Microbiología e Inmunología, Facultad de Medicina Veterinaria y Zootecnia, Universidad Nacional Autónoma de México, Mexico D.F., Mexico.
54p	Evaluation of humoral immune status in porcine epidemic diarrhea virus (PEDV) infected sows under field conditions	K. Ouyang[1], **D. Shyu**[1], S. Dhakal[1], J. Hiremath[1], B. Binjawadagi[1], Y. Lakshmanappa[1], R. Guo[2], R. Ransburgh[2], K. Bondra[1], P. Gauger[3], J. Zhang[3], T. Specht[4], A. Gilbertie[4], W. Minton[4], Y. Fang[2], R. Gourapura[1];1Food Animal Health Research Program (FAHRP), Veterinary Preventive Medicine, The Ohio State University, OARDC, Wooster, OH, USA, 2Diagnostic Medicine and Pathobiology, Kansas State Veterinary Diagnostic Laboratory, Kansas State University, College of Veterinary Medicine, Manhattan, KS, USA, 3Vet Diagnostic & Production Animal Medicine, Iowa State University, Ames, IA, USA, 4Four Star Veterinary Services, Chickasaw, OH, USA.
55p	Hydrogen peroxide inactivation of PRRS virus for vaccine preparation	**Y. Burakova**[1], L. Wang[2], R. Madera[2], J.R. Schlup[1], J. Shi[2];1Department of Chemical Engineering, College of Engineering, Kansas State University, Manhattan, KS, USA, 2Department of Anatomy and Physiology, College of Veterinary Medicine, Kansas State University, Manhattan, KS, USA.
56p	Isolation and characterization of equine pulmonary dendritic cells obtained from lung tissue.	Y. Lee, M. Kiupel, G.S. Hussey; Pathobiology & Diagnostic Investigation, Michigan State University, East Lansing, MI, USA.
57p	Early weaning stress in pigs alters postnatal enteric neuro-immune development, inducing long-term disease susceptibility.	**C.S. Pohl**[1], J.E. Medland[2], L.L. Edwards[2], K. Bagley[2], A.J. Moeser[1];1Large Animal Clinical Sciences, Michigan State University, East Lansing, MI, USA, 2Population Health & Pathobiology, North Carolina State University, Raleigh, NC, USA.
58p	Predicting relatedness of PRRSv strains based on whole genome T cell epitope content	**A.H. Gutierrez**[1], C. Loving[2], L. Moise[3], W. Martin[4], A.S. De Groot[3];1Institute for Immunology and Informatics, University of Rhode Island, Providence, RI, USA, 2Virus and Prion Diseases Research Unit, NADC, USDA ARS, Ames, IA, USA, 3Institute for Immunology and Informatics, University of Rhode Island; EpiVax Inc., Providence, RI, USA, 4EpiVax Inc., Providence, RI, USA.
59p	BVDV infection significantly compromise the bovine neutrophils activity	N.L.C. Chase; Veterinary and Biomedical Sciences, South Dakota State University, Brookings, SD, USA.
60p	Standardization of an indirect ELISA test from purified protein immunodominant extracts of Brucella canis	**A. Morales-Aguilar**[1], A. Benítez-Guzmán[1], F. Suárez-Güemes[1], E. Díaz-Aparicio[2], E.G. Palomares-Reséndiz[2], B. Arellano-Reynoso[1];1Microbiología e Inmunología, Universidad Nacional Autónoma de México, Ciudad de México, Mexico, 2Laboratorio de Enfermedades de los Pequeños Rumiantes, Instituto Nacional de Investigaciones Forestales, Agrícolas y Pecuarias, Ciudad de México, Mexico.
61p	Borrelia burgdorferi-induced IL-10 expression in C57BL/6 mice is mediated by cyclic-AMP and requires CD14-dependent p38-MAPK activation	**A. Verma**[1], B. Sahay[2], K. Strle[3], R.L. Patsey[2], K. Basant[2], A. Steere[3], T. Sellati[4];1College of Veterinary Medicine, Lincoln Memorial University, Harrogate, TN, USA, 2Albany Medical College, Albany, NY, USA, 3Massachusetts General Hospital, Boston, MA, USA, 4Trudeau Insitute, Saranac Lake, NY, USA.

Poster Session II - Monday 5:00 - 6:30 PM - Grandballroom Salon III - 7th Floor Poster Assembly begins at 12:00 PM Monday. Please remove your poster by 6:30 PM Monday. Poster Presenters must be with their competiton entry posters for possible judge interviews.
Name badge are required.

Immunology/Renukaradhya Gourapura and Radhey Kaushik

No.	Title	Author
62p	US-UK Collaborative Swine Immune Toolkit Initiative: Development of new immune reagents for swine health, vaccine and disease studies.	**J.K. Lunney**[1], M. Bailey[2], A. Crossman[1], G.J. Renukaradhya[3], J.W. LaBresh[4], S. Elankumaran[5], Y. Sang[6], L. Wooldridge[2];1USDA ARS BARC APDL, Beltsville, MD, USA, 2University of Bristol, Bristol, UK, 3The Ohio State University, Wooster, OH, USA, 4Kingfisher Biotech, Inc., St. Paul, MN, USA, 5Virginia Polytechnic Institute and State University, Blacksburg, VA, USA, 6Kansas State University, Manhattan, KS, USA.
63p	Monoclonal antibodies against the spike glycoprotein of porcine epidemic diarrhea virus	S. Lawson, F. Okda, K.S. Hain, L.R. Joshi, A. Singrey, X. Liu, J. Nelson, E.A. Nelson, D.G. Diel; Veterinary and Biomedical Sciences, South Dakota State University, Brookings, SD, USA.
64p	Testing the effectiveness of combinational adjuvants for subunit vaccines	**R. Madera**[1], A. Beckley[1], R. Shrestha[2], K. Jia[2], P. Li[2], J. Shi[1];1Department of Anatomy and Physiology, Kansas State University, Manhattan, KS, USA, 2Department of Chemistry, Kansas State University, Manhattan, KS, USA.
65p	Mycobacterium bovis biomass and culture filtrate protein extracts induce bovine macrophage apoptosis.	A. Maciel-Rivera[1], E. Alfonseca-Silva[1], C. Espitia-Pinzón[2], C. Parada-Colín[2], L. Arriaga-Pizano[3], A. Benítez-Guzmán[1], **J.A. Gutierrez-Pabello**[1];1Laboratorio de Investigación en Tuberculosis y Brucelosis, Facultad de Medicina Veterinaria y Zootecnia, Universidad Nacional Autónoma de México, Mexico City, Mexico, 2Instituto de Investigaciones Biomédicas, Universidad Nacional Autónoma de México, Mexico City, Mexico, 3Unidad Médica de Investigación en Inmunoquímica, Hospital Siglo XXI, Mexico City, Mexico.
66p	Matrix Protein 2 based vaccine protects against swine influenza H1N1virus infection	M. Thomas[1], C. Sreenivasan[1], Z. Wang[1], B. Hause[2], F. Li[3], D. Francis[4], **R. Kaushik**[3];1Dept of Biology and Microbiology, South Dakota State University, Brookings, SD, USA, 2Department of Diagnostic Medicine and Pathobiology, Kansas State University, Manhattan, KS, USA, 3Dept of Biology and Microbiology and Dept. of Veterinary and Biomedical Sciences, South Dakota State University, Brookings, SD, USA, 4Dept. of Veterinary and Biomedical Sciences, South Dakota State University, Brookings, SD, USA.
67p	Novel monoclonal antibodies against the outer membrane protein-31 of Brucella melitensis are potential diagnostic tools.	**P. Luo**[1], Z. He[1], W. Wang[1], Q. Liao[1], J. Wu[1], S. Chen[2], H. Zhang[3], G. Lou[4], J.-P. Allain[5], C. Li[1];1Dept of Transfusion Medicine, Southern Medical University, Guangzhou, China, 2Guangzhou Center of Disease Control and Prevention, Guangzhou, China, 3School of Animal Science and Technology, Shihezi University, Shihezi, China, 4Shaoguan College, Shaoguan, China, 5Department of Hematology, University of Cambridge, Cambridge, UK.

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Immunology/Renukaradhya Gourapura and Radhey Kaushik

68p	Ovine MHC class I and class II DRB1 allele polymorphism associated with cellular immune response in vaccinated sheep with the attenuated Brucella melitensis	**Z. He**[1], P. Luo[1], W. Wang[1], Y. Weng[1], Q. Liao[1], T. Li[1], L. Zhang[1], J. Wu[1], G. Lou[2], J.-P. Allain[3], C. Li[1];1Dept of Transfusion Medicine, Southern Medical University, Guangzhou, China, 2Henry Fok Institute for Animal Diseases, Shaoguan College, Shaoguan, China, 3Department of Hematology, University of Cambridge, Cambridge, UK.
069p	Characterization of immunological gene expression in the intestine of healthy calves	K.H. Wade, E. Bichi, J.F. Lowe, B.M. Aldridge; College of Veterinary Medicine, University of Illinois, Urbana, IL, USA.
070p	Comparison of serum PTX3 from shipping through sickness and recovery in cattle	**C.G. Chitko-McKown**[1], A.M. Workman[1], K.D. DeDonder[2], G.L. Bennett[1], M.D. Apley[2], G.P. Harhay[1], D.M. Harhay[3], L.A. Kuehn[1], B.J. White[2], R.L. Larson[2], S.F. Capik[2], B.V. Lubbers[2], M.L. Clawson[1];1Genetics, Breeding, & Animal Health Research Unit, U.S. Meat Animal Research Center, Clay Center, NE, USA,
071p	Comparative analysis of signature genes in PRRSV-infected porcine monocyte-derived dendritic cells at differential activation statuses.	**L.C. Miller**[1], Y. Sang[2], F. Blecha[2];1VPDRU, USDA-ARS-NADC, Ames, IA, USA, 2Departments of Anatomy and Physiology, College of Veterinary Medicine, Kansas State University, Manhattan, KS, USA.

Respiratory Diseases/Amelia Woolums

No.	Title	Author
072p	Utilization of a behavior score to detect bovine respiratory disease in preweaned group-housed calves	M.C. Cramer, A.L. Stanton; Department of Dairy Science, University of Wisconsin-Madison, Madison, WI, USA.
073p	Genetic diversity and antigenic characterization of Quebec influenza virus strains isolated from pigs	Z. Mhamdi, C. Savard, C.A. Gagnon; Groupe de recherche sur les maladies infectieuses du porc (GREMIP), Swine and Poultry Infectious Diseases Research Centre (CRIPA); Faculty of Veterinary Medicine, University of Montreal, Saint-Hyacinthe, QC, Canada.
074p	Development of fully-automated DNA microarray-chips for multiplex detection of bovine pathogens	**N. Thanthrige-Don**[1], T. Furukawa-Stoffer[2], C. Buchanan[2], T. Joseph[2], D. Godson[2], T. Alexander[5], O. Lung[2], A. Ambagala[2];1Faculty of Veterianry Medicine, University of Calgary, Calgary, AB, Canada, 2National Centres for Aniimal Disease, Canadian Food Inspection Agency, Lethbridge, AB, Canada, 3Animal Health Centre, Ministry of Agriculture, Abbotsford, BC, Canada, 4Prairie
075p	Fostera®PRRS vaccine helps provide heterologous cross-protection against a wide range of diverse Porcine Reproductive and Respiratory Syndrome viruses	**J.G. Calvert**[1], J. Angulo[2], J.R.D. Allison[3], B. O'Brien[2], R.G. Ankenbauer[1];1Veterinary Medicine R&D, Zoetis Inc, Kalamazoo, MI, USA, 2Veterinary Operations Pork, Zoetis Inc, Florham Park, NJ, USA, 3Corporate Development/ Life Cycle Management, Zoetis Inc, Florham Park, NJ, USA.

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Respiratory Diseases/Amelia Woolums

No.	Title	Author
078p	Use of matrix-assisted laser desorption ionization time-of-flight mass spectrometry to identify reclassified species, Streptococcus parasuis	**L. Marshall Lund**[1], L. McDeid[2], A. Chriswell[2], K. Harmon[2], T. Frana[1];1Veterinary Diagnostic and Production Animal Medicine, Iowa State University, Ames, IA, USA, 2Veterinary Diagnostic Laboratory, Iowa State University, Ames, IA, USA.

Vector-Borne and Parasitic Diseases/Roman Ganta

No.	Title	Author
079p	Analysis of intra-host genetic diversity in Rift Valley fever virus infection of ruminants	**V. Shivanna**[1], C. McDowell[1], D.C. Jasperson[2], N.N. Gaudreault[2], A.S. Davis[1], B. Faburay[1], I. Morozov[1], W.C. Wilson[2], J.A. Richt[1];1Department of Diagnostic Medicine/Pathobiology, Kansas State University, Manhattan, KS, USA, 2Arthropod Borne Animal Disease Research Unit, United States Department of Agriculture, Agricultural Research Service, Manhattan, KS, USA.

Viral Pathogeneses/Kyoung-Jin Yoon

080p	Porcine deltacoronavirus induces apoptosis in swine testicular and LLC porcine kidney cell lines in vitro but not in infected intestinal enterocytes in vivo	K. Jung, H. Hu, L.J. Saif; Food Animal Health Research Program, Ohio State University, Wooster, OH, USA.
081p	Stress-activated protein kinases are involved in porcine epidemic diarrhea virus infection	Y. Kim, J. Jeon, C. Lee; Kyungpook National University, Daegu, Korea, Republic of.
082p	Genetic analysis of a pathogenic Korean PEDV strain at different passage levels	S. Lee, C. Lee; Kyungpook National University, Daegu, Korea, Republic of.
083p	Mutations in the genome of a virulent porcine reproductive and respiratory syndrome virus nsp2 deletion strain associated with attenuation	S.-C. Lee[1], **S. Lee**[2], H.-W. Choi[1], I.-J. Yoon[1], S.-Y. Kang[3], C. Lee[2];1Choongang Vaccine Laboratory, Daejeon, Korea, Republic of, 2Kyungpook National University, Daegu, Korea, Republic of, 3Chungbuk National University, Daejeon, Korea, Republic of.
084p	Proteolytic processing of porcine reproductive and respiratory syndrome virus replicase orf1a polyprotein	**Y. Li**[1], A. Tas[2], Z. Sun[1], E.J. Snijder[2], Y. Fang[1];1Diagnostic Medicine / Pathobiology, Kansas State university, Manhattan, KS, USA, 2Department of Medical Microbiology, Center for Infectious Diseases, Leiden University Medical Center, Leiden, Netherlands.

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Viral Pathogeneses/Kyoung-Jin Yoon

No.	Title	Author
087p	Epidemiological situation of African swine fever in Ukraine in 2015	O. Nevolko; Virology, Institute of Laboratory Diagnostics (SSRILDVSM), Kyiv, Ukraine.
088p	Production and characterization of monoclonal antibodies against emerging swine pestivirus	F. Yuan, Z. Chen, Y. Wang, P. Shang, B. Hause, Y. Fang; Diagnostic Medicine/Pathobiology (DMP), Kansas State University, Manhattan, KS, USA.

ORAL

PROGRAM

Bacterial Pathogenisis Avenue Ballroom - 4th Floor Section Leader: Gireesh Rajashekara			
TIME	**NO**	**TITLE**	**AUTHORS**
8:00	1	Re-evaluating the LD50 requirements in the codified potency testing of Leptospira in the United States	**A. Walker**, R. Olsen, L. Ludemann, G. Srinivas; Center for Veterinary Biologics, National Centers for Animal Health, Ames, IA, USA.
8:15	2	Dynamic attachment of differentially expressed Actinobacillus suis adhesins to host extracellular matrix components	**A.R. Bujold**, J.I. MacInnes; Pathobiology, University of Guelph, Guelph, ON, Canada.
8:30	3	Direct enrichment and de novo sequencing of Mycobacterium avium subspecies paratuberculosis from fecal samples of cattle for genomic epidemiology applications	**F.M. Shoyama**, S. Wells, S. Sreevatsan; Veterinary Population Medicine, University of Minnesota, Saint Paul, MN, USA.
8:45	4	Improved diagnostics of Mycobacterium tuberculosis complex infections in Minnesota white-tailed deer	**S.I. Wanzala**[1], M. Carstensen[2], R. Waters[3], S. Sreevatsan[1]; [1]Veterinary Population Medicine, University of Minnesota, Saint Paul, MN, USA, [2]Department of Natural Resources, Greater Minneapolis-Saint Paul Area, MN, USA, [3]National Animal Disease Center, USDA, Ames, IA, USA.
9:00	5	High throughput screening to identify quorum sensing inhibitors to enhance the control of avian pathogenic e. coli	**Y.A. Helmy**, I.I. Kassem, A. Kumar, L. Deblais, G. Rajashekara; Food Animal Health Research Program, Department of Veterinary Preventive Medicine, Ohio Agricultural Research and Development Center, Ohio State University, Wooster, OH, USA.
9:15	6	High-throughput screening to identify novel anti-campylobacter compounds using a pre-selected enriched small molecules library	**A. Kumar**[1], R. Pina-Mimbela[1], X. Xuilan Xu[2], J. Fuchs[3], C. Nislow[4], J. Templeton[5], P.J. Blackall[6], G. Rajashekara[1]; [1]Food Animal Helath Research Program, The Ohio State University, Wooster, OH, USA, [2]Department of Plant Pathology, The Ohio State University, Wooster, OH, USA, [3]College of Pharmacy, The Ohio State University, Columbus, OH, USA, [4]Pharmaceutical Sciences, University of British Columbia, Vancouver, BC, Canada, [5]Department of Agriculture and Fisheries, EcoSciences Precinct, The University of Queensland, Queensland, Australia, [6]5Department of Agriculture and Fisheries, EcoSciences Precinct,, The University of Queensland, Duton Park, Queensland, Australia.

Bacterial Pathogenisis Avenue Ballroom - 4th Floor Section Leader: Gireesh Rajashekara			
TIME		**TITLE**	**AUTHORS**
9:30		**Break and Table Top Exhibits - Foyer**	
10:00	7	A genomics-enabled 'sexual hybridization' strategy directly identifies the genetic basis for emergent virulence caused by a rapidly expanding clone of Campylobacter jejuni	**Z. Wu**[1], O. Sahin[1], M. Yaeger[2], B. Periaswamy[3], S. Chen[3], V. Lashley[2], P. Plummer[4], Z. Shen[1], Q. Zhang[1]; [1]Department of Veterinary Microbiology and Preventive Medicine, Ames, IA, USA, [2]Department of Veterinary Pathology, Ames, IA, USA, [3]Genome Institute of Singapore, Singapore, Singapore,[4]4.Department of Veterinary Diagnostic and Production Animal Medicine, Ames, IA, USA.
10:15	8	Identification and characterization of virulence factors of staphylococcus aureus isolates from cases of bovine mastitis	**O. Kerro Dego**, R. Almeida, S. Oliver; Animal Science, The University of Tennessee, Knoxville, TN, USA.
10:30	9	Genetic mechanisms of Salmonella enterica serovar Typhimurium for overcoming host stressors	**R.K. Mandal**, Y.M. Kwon; Poultry Science, University of Arkansas, Fayetteville, AR, USA.
10:45	10	Driving through the fog: Understanding type A Clostridium perfringens in enteric disease of animals	**J.F. Prescott**; Department of Pathobiology, University of Guelph, Ontario, ON, Canada.
11:30		**Lunch**	
1:00	11	Our specific objectives were to examine the role of NetB in pathogenesis and the true place of alpha toxin (CPA), if any, in disease development.	**J.G. Songer**[1], C.C. Boyiddle[2], M.A. Anderson[3], K.K. Cooper[4]; [1]Iowa State University, Ames, IA, USA, [2]Ventana Medical Systems, Tucson, AZ, USA, [3]University of Arizona, Tucson, AZ, USA, [4]California State University, Northridge, CA, USA.
1:45	12	Clostridium difficile infection - A One Health problem.	**J. Scaria**; Veterinary and Biomedical Science, South Dakota State University, Brookings, SD, USA.
2:15	13	Carvacrol reduces Clostridium difficile virulence without inducing gut dysbiosis	**K. Venkitanarayan**; Department of Animal Science, University of Connecticut, Storrs, CT, USA.
2:45		**Break and Table Top Exhibits - Foyer**	
3:00	14	Biofilms, wounds, and chronic infections	**D. Wozniak**; Microbial Infection and Immunity Microbiology, Ohio State University, Columbus, OH, USA.
3:45	15	The Toll-like receptor 2 (TLR2) does not play a critical role during systemic and central nervous system infections by Streptococcus suis strains from different backgrounds	**J.-P. Auger**, M. Segura, M. Gottschalk; GREMIP & CRIPA, Faculty of Veterinary Medicine, University of Montreal, Saint-Hyacinthe, QC, Canada.
4:00	16	Interactions between Streptococcus suis and Haemophilus parasuis	A.E. Barre, E.P. Kunkel, **J.I. MacInnes**; Dept. of Pathobiology, University of Guelph, Guelph, ON, Canada.
4:15	17	Biofilm formation by Mannheimia haemolytica on bovine bronchial epithelial cells.	**I. Boukahil**, C.J. Czuprynski; University of Wisconsin-Madison, Madison, WI, USA.

Bacterial Pathogenisis Avenue Ballroom - 4th Floor Section Leader: Gireesh Rajashekara			
4:30	18	Mannheimia haemolytica biofilm cells induce neutrophil extracellular trap (NET) formation in vitro	**N.A. Aulik**[1], R. Matulle[2], C. Czuprynski[2]; [1]Wisconsin Veterinary Diagnostic Laboratory, University of Wisconsin-Madison, Madison, WI, USA, [2]Department of Pathobiological Sciences, University of Wisconsin-Madison, Madison, WI, USA.
5:00	**6:30**	**Poster Session II Grand Ballroom Salon III - 7th Floor**	

Biosafey And Biosecurity Northwestern/Ohio - 6th Floor Section Leader:Brandy Burgess			
TIME	**NO**	**TITLE**	**AUTHORS**
1:30	19	Characteristics of infection control practices at North American veterinary teaching hospitals	**C.S. De La Hoz Ulloa**[1], K.M. Benedict[2], P.S. Morley[2], B.A. Burgess[1]; [1]Department of Population Health Sciences, Virginia Maryland College of Veterinary Medicine, Blacksburg, VA, USA, [2]Department of Clinical Sciences, Colorado State University, Fort Collins, CO, USA.
1:45	20	Evaluating environmental sampling methods for detection of S. enterica in a large animal veterinary hospital	**V. Goeman**, S.H. Tinkler, G.K. Hammac, A. Ruple-Czerniak; Purdue University College of Veterinary Medicine, West Lafayette, IN, USA.
2:00	21	The importance of being clean: biosecurity measures in farm operations.	**G. Rossi**[1], R. Smith[1], S. Pongolini[2], S. Natalini[3], L. Bolzoni[2]; [1]Pathobiology, University of Illinois, Urbana, IL, USA, [2]Sezione di Parma, IZSLER, Parma, Italy, [3]Servizio Veterinario ASL, Regione Emilia Romagna, Bologna, Italy.
2:15	22	Simultaneous detection of swine respiratory pathogens using a multiplexed detection assay	**C. Carrillo**[1], J. Olivas[1], P. Naraghi-Arani[1], S. Gardner[1], R. Ransburgh[2], X. Liu[2], J. Bai[2], Y. Fang[2], R.R.R. Rowland[2], C. Jaing[1]; [1]Physical & Life Sciences Directorate, Lawrence Livermore National Laboratory, Livermore, CA, USA, [2]Diagnostic Medicine & Pathobiology Department, Kansas State University, Manhattan, KS, USA.
2:30	23	Rapid detection of foot-and-mouth disease virus using a field-deployable, reverse-transcription insulated-isothermal PCR assay	**A. Ambagala**[1], M. Fisher[1], M. Goolia[2], C. Nfon[2], T. Furukawa-Stoffer[1], P. Lee[3], R.O. Polo[1], O. Lung[1]; [1]National Centres for Aniimal Disease, Canadian Food Inspection Agency, Lethbridge, AB, Canada, [2]National Centres for Aniimal Disease, Canadian Food Inspection Agency, Winnipeg, MB, Canada,[3]GeneReach USA, Lexington, MA, USA.
2:45		**Break and Table Top Exhibits - Foyer**	
3:00	24	Infectious disease transmission risks on livestock farms: biosecurity practices, dogs and education	**J. Stull**; Department of Veterinary Preventive Medicine, Ohio State University, Columbus, OH, USA.
3:45	25	Development of an animal model for Schmallenberg virus	**A.D. Endalew**[1], B. Bawa[2], N. Gaudreault[1], M. Ruder[3], B. Drolet[3], S. Mcvey[3], W. Ma[1], I. Morozov[1], B. Faburay[1], W. Wilson[3], J. Richt[1]; [1]Diagnostic Medicine and Pathobiology, Kansas State University, Manhattan, KS, USA, [2]Abbvie Inc., Chicago, IL, USA, [3]Arthropod-Borne Animal Diseases Research Unit, USDA-ARS, Manhattan, KS, USA.
4:00	26	Polymorphism analysis of prion protein gene in eleven pakistani goat breeds	**M.F. HASSAN**; STATE KEY LAB OF TSE, VETERINARY PATHOLOGY, CHINA AGRICULTURAL UNIVERSITY, BEIJING, China.
4:45		Break and Table Top Exhibits - Foyer	
5:00	**6:30**	**Poster Session II Grand Ballroon Salon III - 7th Floor**	

Companion Animals Epidemiology
Salon E - 5th Floor
Section Leader Audrey Ruple-Czerniak & Laura Hungerford

TIME	NO.	TITLE	AUTHORS
8:00	27	Burden and predictors of Staphylococcus spp. infections among dogs presented at a veterinary academic hospital in South Africa	**N.D. Qekwana**, J.W. Oguttu, A. Odoi; Section Veterinary Public Health, Department off Paraclinical Sciences, University of Pretoria, Pretoria, South Africa, Section: Animal Health, Department of Agriculture and Animal Health, University of South Africa, Pretoria, South Africa, Biomedical and Diagnostic Sciences College of Veterinary Medicine, University of Tennessee, Knoxville, TN, USA.
8:15	28	Saving Fido - Unearthing a novel topical antimicrobial for treatment of multidrug-resistant staphylococcal skin infections in companion animals	**H. Mohammad**[1], A.S. Mayhoub[2], P.V.M. Reddy[2], M. Cushman[2], M. Seleem[1]; [1]Comparative Pathobiology, Purdue University, West Lafayette, IN, USA, [2]Medicinal Chemistry and Molecular Pharmacology, Purdue University, West Lafayette, IN, USA.
8:30	29	A field-deployable POCKIT™ Nucleic Acid Detection System for specific and sensitive point-of-need detection of feline leukemia virus RNA and proviral DNA	**R.P. Wilkes**[1], E. Anis[1], P.-Y. Lee[2], F.-C. Lee[2], Y.-L. Tsai[2], H.-F.G. Chang[2], H.-T.T. Wang[2]; [1]Veterinary Diagnostic and Investigational Laboratory, The University of Georgia, Tifton, GA, USA, [2]GeneReach USA, Lexington, MA, USA.
8:45	30	Temporal trends of feline retroviral infections diagnosed at the Ontario Veterinary College (1999-2012)	B. Chhetri[1], **O. Berke**[1], D. Pearl[1], B. McEwan[2], D. Bienzle[3]; [1]Population Medicine, University of Guelph, Guelph, ON, Canada, [2]Animal Health Laboratory, University of Guelph, Guelph, ON, Canada, [3]Pathobiology, University of Guelph, Guelph, ON, Canada.
9:00	31	Antimicrobial resistance in Escherichia coli isolated from equine clinical diagnostic specimens	**A.S. Rogovskyy**, C. Gillis; Veterinary Pathobiology, College of Veterinary Medicine and Biomedical Sciences, Texas A&M University, College Station, TX, USA.
9:15	32	Utility of electronic medical record data for healthcare-associated infection detection with fever sequella	**Z.B. Ouyang**[1], B. Burgess[2], P. Morley[1]; [1]Colorado State University, Fort Collins, CO, USA, [2]Virginia Tech, Blacksburg, VA, USA.
9:30		**Break and Table Top Exhibits - Foyer**	
10:00	33	GRADE approach as a tool for making evidence-based decisions in veterinary medicine	**B.L. Robinson**, L.L. Hungerford; Office of New Animal Drug Evaluation, U.S. Food and Drug Administration, Center for Veterinary Medicine, Rockville, MD, USA.
10:15	34	Frequency, benefits and risks surrounding animals in Ohio nursing home facilities	**J. Stull**[1], C. Hoffman[2], T.F. Landers[3]; [1]Department of Veterinary Preventive Medicine, Ohio State University, Columbus, OH, USA, [2]College of Public Health, Ohio State University, Columbus, OH, USA, [3]College of Nursing, Ohio State University, Columbus, OH, USA.

Companion Animals Epidemiology
Salon E - 5th Floor
Section Leader Audrey Ruple-Czerniak & Laura Hungerford

TIME	NO.	TITLE	AUTHORS
10:30	35	Epidemiologic aspects of fecal Salmonella and Campylobacter shedding among dogs at seven animal shelters across Texas	**L.D. Rodriguez-Rivera**[1], K.J. Cummings[1], A.M. Leahy[1], S.C. Rankin[2], S.D. Lawhon[1], C.-Y. Yang[1], S.A. Hamer[1]; [1]Veterinary Integrative Biosciences, Texas A&M University, College Station, TX, USA, [2]School of Veterinary Medicine, University of Pennsylvania, Philadelphia, PA, USA.
10:45	36	An observational study of the prevalence of heartworm disease in Mississippi shelter dogs and test efficacy	A. Parisi[1], U. Donnett[1], C. Loftin[1], M. Wang[2], D. Smith[2], **K. Woodruff**[1]; [1]Clinical Sciences, Mississippi State University College of Veterinary Medicine, Mississippi State, MS, USA, [2]Pathobiology and Population Medicine, Mississippi State University College of Veterinary Medicine, Mississippi State, MS, USA.
11:00	37	Progression of surgical efficiency, incision length and complication rate in senior veterinary students enrolled in a 2 week spay/neuter surgical elective	**K.A. Woodruff**[1], U. Donnett[1], J. Shivley[1], D. Smith[2]; [1]Clinical Sciences, Mississippi State University College of Veterinary Medicine, Mississippi State, MS, USA, [2]Pathobiology and Population Medicine, Mississippi State University College of Veterinary Medicine, Mississippi State, MS, USA.
11:15	38	Peri-operative morbidity and risk factors associated with routine sterilization surgeries performed on a mobile surgical service	**U. Donnett**, K. Woodruff, J. Shivley; Veterinary Clinical Sciences, Mississippi State University, Starkville, MS, USA.
11:30		**Lunch**	
4:30		**Break and Table Top Exhibits - Foyer**	
5:00	**6:30**	**Poster Session II Grand Ballroom Salon III - 7th Floor**	

Ecology and Management of Foodborne Agents
Salon E - 5th Floor
Section Leader: Tom Wittum

TIME	NO	TITLE	AUTHORS
11:30		Lunch	
1:30	39	Prevalence and characterization of Salmonella isolated from feral pigs throughout Texas	**K.J. Cummings**[1], L.D. Rodriguez-Rivera[1], M.K. Grigar[1], S.C. Rankin[2], B.T. Mesenbrink[3], B.R. Leland[3], M.J. Bodenchuk[3]; [1]Veterinary Integrative Biosciences, Texas A&M University, College Station, TX, USA, [2]Pathobiology, University of Pennsylvania, Philadelphia, PA, USA, [3]Wildlife Services, United States Department of Agriculture, San Antonio, TX, USA.
1:45	40	Dissemination of carbapenem-resistant Enterobacteriaceae from a municipal wastewater treatment plant	**D.M. Stuever**[1], C.A. King[2], D.F. Mollenkopf[2], D.A. Mathys[2], S. Feicht[2], J.B. Daniels[3], T.E. Wittum[2]; [1]College of Public Health, The Ohio State University, Columbus, OH, USA, [2]Veterinary Preventive Medicine, The Ohio State University, Columbus, OH, USA, [3]Veterinary Clinical Sciences, The Ohio State University, Columbus, OH, USA.
2:00	41	Effect of copper, zinc, and essential oil supplementation on antimicrobial resistance of fecal Escherichia coli in nursery piglets	K. Rozas[1], **R.G. Amachawadi**[2], K. Norman[1], J. Vinasco[1], R. Pugh[1], F. Lopez Perez[1], A. Wakil[1], D. Manriquez[1], M. Tokach[3], T. G. Nagaraja[2], H. M. Scott[1]; [1]Veterinary Pathobiology, Texas A&M University, College Station, TX, USA, [2]Diagnostic Medicine/Pathobiology, Kansas State University, Manhattan, KS, USA, [3]Animal Sciences & Industry, Kansas State University, Manhattan, KS, USA.
2:15	42	Antimicrobial susceptibility of enteric Gram-negative facultative anaerobe bacilli in aerobic versus anaerobic conditions	Z. DeMars, S. Biswas, R. Amachawadi, D. Renter, **V. Volkova**; Department of Diagnostic Medicine/Pathobiology, College of Veterinary Medicine, Kansas State University, Manhattan, KS, USA.
2:45		**Break and Table Top Exhibits - Foyer**	
3:00	43	Comparing the resistome of poultry, swine, cattle and salmon production and nearby human waste water treatment plants	**N. Noyes**[1], M. Weinroth[1], S. Lakin[1], E. Doster[1], R. Raymond[1], P. Rovira-Sanz[1], Z. Abdo[1], J. Ruiz[1], J. Martin[1], C. Boucher[1], K. Jones[2], K.E. Belk[1], P.S. Morley[1]; [1]Colorado State University, Fort Collins, CO, USA, [2]University of Colorado Denver, Denver, CO, USA.
3:15	44	Use of shotgun metagenomic to evaluate the microbiome in cattle feces following tulathromycin metaphylaxis	E. Doster[1], **P. Rovira**[2], N.R. Noyes[1], B.A. Burgess[3], X. Yang[2], M. Weinroth[2], L. Linke[1], R. Magnuson[1], K. Jones[4], C. Boucher[5], J. Ruiz[5], P.S. Morley[1], K.E. Belk[2]; [1]Clinical Sciences, Colorado State University, Fort Collins, CO, USA, [2]Animal Sciences, Colorado State University, Fort Collins, CO, USA,[3]Population Health Sciences, Virginia Tech, Blacksburg, VA, USA, [4]Biochemistry and Molecular Genetics, University of Colorado, Denver, CO, USA, [5]Computer Sciences, Colorado State University, Fort Collins, CO, USA.

Ecology and Management of Foodborne Agents
Salon E - 5th Floor
Section Leader: Tom Wittum

3:30	45	Characterizing variation in the microbial resistome between natural and conventional beef operations	R. Zaheer[1], **S.M. Lakin**[2], N.R. Noyes[2], S. Cook[1], S.P. Gow[3], C.W. Booker[4], S.J. Hannon[4], L. Tymensen[5], J.T. McClure[6], K.E. Belk[2], P.S. Morley[2], T.A. McAllister[1]; [1]Agriculture and Agri-Food Canada, Lethbridge, AB, Canada, [2]Colorado State University, Fort Collins, CO, USA, [3]Public Health Agency of Canada, Saskatoon, SK, Canada, [4]Feedlot Health Management Services, Okotoks, AB, Canada, [5]Alberta Agriculture and Rural Development, Lethbridge, AB, Canada, [6]University of Prince Edward Island, Charlottetown, PE, USA.
3:45	46	Repeated oral immunization with Shiga toxin negative Escherichia coli O157:H7 transiently reduces carriage of wild-type EHEC O157 by cattle following oral challenge	S. Shringi[1], H. Sheng[2], C.E. Schmidt[3], K.N.K. Baker[3], C.J. Hovde[2], S.A. Minnich[2], **T.E. Besser**[4]; [1]Washington State Univesity, Pullman, WA, USA, [2]Bi-state School of Food Science, University of Idaho, Moscow, ID, USA, [3]VMP, Washington State Univesity, Pullman, WA, USA, [4]Department of Veterinary Microbiology and Pathology, Washington State Univesity, Pullman, WA, USA.
4:00	48	Risk profile and quantitative exposure assessment of hepatitis E virus from pigs or	**B.J. Wilhelm**[1], A. Fazil[2], A. Rajic[3], A. Houde[4], S.A. McEwen[1]; [1]Department of Population Medicine, University
4:30	5:00	**Break and Table Top Exhibits - Foyer**	
5:00	6:30	**Poster Session II Grand Ballroom Salon III - 7th Floor**	

Epidemiology and Animal Health Economics
Salon A/B/C/D - 5 th Floor
Section Leader: Ashley Hill

TIME	NO	TITLE	AUTHORS
8:00	50	Trends in antimicrobial resistance patterns of common Salmonella serotypes isolated from bovine samples in Wisconsin from 2006-2015	**J.R. Valenzuela**[1], M.N. Glines[1], D. Lee[2], A.K. Sethi[3], N.A. Aulik[4], K.P. Poulsen[5]; [1]Master in Public Health, University of Wisconsin-Madison, Madison, WI, USA, [2]Wisconsin Veterinary Diagnostic Laboratory, Madison, WI, USA, [3]Department of Population Health Sciences, University of Wisconsin-Madison, Madison, WI, USA, [4]Department of Pathobiological Sciences, University of Wisconsin-Madison, Madison, WI, USA, [5]Medical Sciences Department, University of Wisconsin-Madison, Madison, WI, USA.
8:15	51	Temporal and geo-spatial characterization of Salmonella enterica serotypes isolated in Wisconsin from 2006 to 2015	**M.N. Glines**[1], J.R. Valenzuela[1], K.P. Poulsen[2], N.A. Aulik[3]; [1]Master of Public Health Program, University of Wisconsin-Madison, Madison, WI, USA, [2]Wisconsin Veterinary Diagnostic Laboratory, Medical Science Department, University of Wisconsin-Madison, Madison, WI, USA, [3]Wisconsin Veterinary Diagnostic Laboratory, Department of Pathobiological Sciences, University of Wisconsin-Madison, Madison, WI, USA.
8:30	52	Genotypic characterization of extended-spectrum cephalosporin resistant nontyphoidal Salmonella from the NAHMS Feedlot 2011 study	**D.F. Mollenkopf**[1], D.A. Mathys[1], D.A. Dargatz[2], M.M. Erdman[3], J.B. Daniels[4], T.E. Wittum[1]; [1]Veterinary Preventive Medicine, Ohio State University, Columbus, OH, USA, [2]Centers for Epidemiology and Animal Health, USDA:APHIS:VS, Fort Collins, CO, USA, [3]USDA:APHIS:VS, Ames, IA, USA,[4]Veterinary Clinical Science, Ohio State University, Columbus, OH, USA.
8:45	53	Extended-spectrum cephalosporin and fluoroquinolone-resistant enterobacteriaceae in human and veterinary hospital environments	**D.A. Mathys**[1], D.F. Mollenkopf[1], S. Feicht[1], J.B. Daniels[2], T.E. Wittum[1]; [1]Department of Veterinary Preventive Medicine, College of Veterinary Medicine, Ohio State University, Columbus, OH, USA, [2]Department of Veterinary Clinical Sciences, College of Veterinary Medicine, Ohio State University, Columbus, OH, USA.
9:00	54	Spatial clustering of cefotaxime and ciprofloxacin resistant E. coli among dairy cattle relative to the European starling night roosts	**G.A. Medhanie**[1], D. Pearl[1], S. McEwen[1], M. Guerin[1], C. Jardine[2], J. Schrock[3], J. LeJeune[3]; [1]Population Medicine, University of Guelph, Guelph, ON, Canada, [2]Pathobiology, University of Guelph, Guelph, ON, Canada, [3]The Ohio State University, Wooster, OH, USA.
9:15	55	A survey of case-control studies in veterinary science	**J.N. Cullen**[1], K.M. Makielski[2], J.M. Sargeant[3], A.M. O'Connor[1]; [1]Department of Veterinary Diagnostic and Production Animal Medicine, Iowa State University, Ames, IA, USA, [2]Department of Veterinary Clinical Sciences, Iowa State University, Ames, IA, USA, [3]Department of Population Medicine, University of Guelph, Guelph, ON, Canada.

Epidemiology and Animal Health Economics
Salon A/B/C/D - 5 th Floor
Section Leader: Ashley Hill

9:30		**Break and Table Top Exhibits - Foyer**	
10:00	56	Risk of a first and recurrent case of pathogen specific clinical mastitis	**E. Cha**[1], J. Hertl[2], Y. Schukken[3], L. Tauer[2], F. Welcome[2], Y. Gröhn[2]; [1]Kansas State University, Manhattan, KS, USA, [2]Cornell University, Ithaca, NY, USA, [3]GD Animal Health, Deventer, Netherlands.
10:15	57	Pregnancy loss attributable to mastitis in first lactation Holstein cows	**M. Dahl**[1], F. Maunsell[1], A. De Vries[2], K. Galvao[1], C. Risco[1], J. Hernandez[1]; [1]College of Veterinary Medicine, University of Florida, Gainesville, FL, USA, [2]Animal Sciences, University of Florida, Gainesville, FL, USA.
10:30	58	The value of pathogen information in treating clinical mastitis.	**E. Cha**[1], R. Smith[2], A. Kristensen[3], J. Hertl[4], Y. Schukken[5], L. Tauer[4], F. Welcome[4], Y. Gröhn[4]; [1]Kansas State University, Manhattan, KS, USA, [2]University of Illinois, Urbana, IL, USA, [3]University of Copenhagen, Copenhagen, Denmark, [4]Cornell University, Ithaca, NY, USA, [5]GD Animal Health, Deventer, Netherlands.
10:45	59	The effect of calf gender and age of dam on the risk for calves to develop bovine respiratory disease prior to weaning	**M. Wang**, K.A. Barton, L.G. Schneider, A.M. Reeves, D.R. Smith; College of Veterinary Medicine, Mississippi State University, Starkville, MS, USA.
11:00	60	An update and model assessment of a mixed treatment comparison meta-analysis of antibiotic treatment for bovine respiratory disease	A.M. O'Connor[1], C. Yuan[2], **J.N. Cullen**[1], N. da Silva[2], J.F. Coetzee[1], C. Wang[2]; [1]Department of Veterinary Diagnostic and Production Animal Medicine, Iowa State University, Ames, IA, USA, [2]Department of Statistics, Iowa State University, Ames, IA, USA.
11:15	61	An investigation of piglet iron status at weaning and subsequent post-weaning growth performance	**A.M. Perri**[1], R. Friendship[1], J.C.S. Harding[2], T.L. O'Sullivan[1]; [1]Population Medicine, University of Guelph, Guelph, ON, Canada, [2]Large Animal Clinical Sciences, University of Saskatchewan, Saskatoon, SK, Canada.
11:30		**Lunch**	
1:30	62	A transboundary, epidemiologic simulation model for the spread and control of classical swine fever among commercial swine in the United States and Canada.	**M.L. Meyer**[1], T.C. Boyer[2]; [1]Division of Environmental Health Sciences, School of Public Health, University of Minnesota, Minneapolis, MN, USA, [2]National Center for Food Protection and Defense, Saint Paul, MN, USA.
1:45	63	Quantification of strategies to mitigate animal-welfare concerns due to movement restrictions during a classical swine fever (CSF) outbreak	**S. Yadav**, H.-Y. Weng; Comparative Pathobiology, Purdue University, West Lafayette, IN, USA.
2:00	64	Risk ranking of irish salmon farms based on network metrics and biosecurity evaluation	**T. Yatabe**[1], S.J. More[2], F. Geoghegan[3], C. McManus[4], A.J. Hill[1], B. Martinez-Lopez[1]; [1]University of California Davis, Davis, CA, USA, [2]University College Dublin, Dublin, Ireland, [3]Marine Institute, Oranmore, Ireland, [4]Marine Harvest Ireland, Rinmore, Ireland.
2:15	65	Modeling the transmission of Orf in order to identify the mechanisms causing persistence.	**H.E. Burson**[1], R.L. Smith[2]; [1]Mathematics, University of Illinois at Urbana Champaign, Urbana, IL, USA, [2]Pathobiology, College of Veterinary Medicine, University of Illinois at Urbana Champaign, Urbana, IL, USA.

Epidemiology and Animal Health Economics Salon A/B/C/D - 5 th Floor Section Leader: Ashley Hill			
2:30	66	Filling gaps in notification data: a model-based approach applied to travel related campylobacteriosis cases in New Zealand	**E. Amene**[1], B. Horn[2], R. Pirie[3], R. Lake[3], D. Döpfer[1]; [1]Medical Sciences, University of Wisconsin-Madison, Madison, WI, USA, [2]Medical Sciences, Institute of Environmental Science and Research Limited, Christchurch, New Zealand, [3]Institute of Environmental Science and Research Limited, Christchurch, New Zealand.
2:45		**Break and Table Top Exhibits - Foyer**	
3:00	67	Role of carriers in the transmission dynamics of bighorn sheep pneumonia	**B. Raghavan**[1], P. Wolff[2], A. Kugadas[1], S. Batra[1], J. Bavananthasivam[1], S. Shanthalingam[1], W.J. Foreyt[1], M.W. Miller[3], S. Subramaniam[1]; [1]Veterinary Microbiology & Pathology, Washington State University, Pullman, WA, USA, [2]Nevada Department of Wildlife, Reno, NV, USA, [3]Wildlife Health Program, Colorado Parks & Wildlife, Fort Collins, CO, USA.
3:15	68	Developing sampling guidelines for oral fluid-based PRRSV surveillance	**M. Rotolo**[1], L. Gimenez-Lirola[1], Y. Sun[1], S. Abate[2], C. Wang[1], D. Baum[1], P. Gauger[1], M. Hoogland[3], R. Main[2], J. Zimmerman[1]; [1]Veterinary Diagnostic and Production Animal Medicine, Iowa State University, Ames, IA, USA, [2]Veterinary Diagnostic Laboratory, Iowa State University, Ames, IA, USA,[3]Murphy-Brown LLC, Algona, IA, USA.
3:30	69	Detecting human brucellosis in rural Uganda: Comparison of a commercial lateral flow assay with microagglutination on sera from high-risk subjects	**R. Miller**[1], J.L. Nakavuma[2], R.V. Tiller[3], R. Stoddard[3], M. Guerra[3], J.B. Kaneene[1]; [1]Center for Comparative Epidemiology, Michigan State University, East Lansing, MI, USA, [2]Makerere University, Kampala, Uganda, [3]Centers for Disease Control and Prevention, Atlanta, GA, USA.
3:45	70	Comparative study of diagnostic tests for Tuberculosis in cattle	**V.M. Belamaranahally**[1], N. Dwarakacherla[1], S. Metuku[2], R. Doddamane[1]; [1]Microbiology, Veterinary College, BANGALORE, India, [2]IAH VB, BANGALORE, India.
4:00	71	Comparative evaluation of blood based Lateral Flow Assay with other serological tests and Polymerase Chain Reaction for the diagnosis of Brucellosis in livestock	**V.M. Belamaranahally**[1], K.B. Anjaneyareddy[1], R. Shome[2], S.M. Isloor[1], L. Gowda[3], A.C. Kamran[4]; [1]Veterinary Microbiology, Veterinary College, Bangalore, India, [2]ICAR, NIVEDI, Bangalore, India, [3]Veterinary Public Health, Veterinary College, Bangalore, India, [4]Veterinary Medicine, Veterinary College, Bangalore, India.
4:15	72	Detection of Dichelobacter nodosus and Fusobacterium necrophorum from footrot of sheep and goats in Andhra Pradesh: Southern India.	**N. VINOD KUMAR**[1], D. SREENIVASULU[1], A. KARTHIK[1], S. VIJAYA LAKSHMI[2]; [1]Department of Veterinary Microbiology, Cllege of Veterinary Science, Tiurpati, SV Veterinary University, Tirupati AP, 517 502.INDIA., Tirupati, India, [2]Department of Veterinary MIcrobiology, Cllege of Veterinary Science, Tiurpati, SV Veterinary University, Tirupati AP, 517 502.INDIA., Tirupati, India.
TUES. 8:00:00 AM	73	Experiential learning: The Farm to Table Study Program as a case study	**S.J. Wells**; College of Veterinary Medicine, University of Minnesota, St Paul, MN, USA.

Epidemiology and Animal Health Economics Salon A/B/C/D - 5 th Floor Section Leader: Ashley Hill			
8:45	49	Mark Gearhart Memorial Award, Salon A/B/C/D Performance and carcass characteristics of commercial feedlot cattle from a study of vaccine and direct-fed microbial effects on <i>Escherichia coli O157:H7 fecal shedding	**C.A. Cull**, D.G. Renter, N.M. Bello, S.E. Ives, A.H. Babcock; Diagnostic Medicine/Pathobiology, Kansas State University, Manhattan, KS, USA.
9:00	75	Understanding PEDV transmission by live haul transport at swine lairage facilities	**J. Lowe**, K. Duda; Veterinary Clinical Medicine, University of Illinois, Urbana, IL, USA.
9:15	76	Developing sampling guidelines for PEDV surveillance	**M. Rotolo**[1], L. Gimenez-Lirola[1], S. Abate[2], M. Hoogland[3], C. Wang[1], D. Baum[1], P. Gauger[1], K. Harmon[2], R. Main[2], A. Ramirez[1], J. Zimmerman[1]; [1]Veterinary Diagnostic and Production Animal Medicine, Iowa State University, Ames, IA, USA, [2]Veterinary Diagnostic Laboratory, Iowa State University, Ames, IA, USA, [3]Murphy-Brown LLC, Algona, IA, USA.
10:00	77	Mental health and wellness in veterinarian and agricultural producers in Ontario, Canada	**A. Jones-Bitton**[1], C. Best[1], P. Conlon[2], J. Hewson[3]; [1]Department of Population Medicine, University of Guelph, Guelph, ON, Canada, [2]Ontario Veterinary College Dean's Office, University of Guelph, Guelph, ON, Canada, [3]Department of Clinical Studies, University of Guelph, Guelph, ON, Canada.
10:15	78	Comparison of Johne's disease prevalence on organic and conventional dairy farms in Pennsylvania	M.-E. Fecteau[1], R.W. Sweeney[1], H. Karremann[2], T. Fyock[1], **H. Aceto**[1]; [1]Clinical Studies - New Bolton Center, University of Pennsylvania, School of Veterinary Medicine, Kennett Square, PA, USA, [2]Rodale Institute, Kutztown, PA, USA.
10:30	79	Meta-analysis of the effects of laidlomycin propionate or monensin sodium on performance, health, and carcass outcomes in finishing steers in North America	**N. Cernicchiaro**[1], M. Quinn[2], M. Corbin[2], D.G. Renter[1]; [1]Diagnostic Medicine and Pathobiology, Kansas State University, Manhattan, KS, USA, [2]Zoetis, Florham Park, NJ, USA.
10:45	80	Cluster analysis of Campylobacter isolates obtained from beef cattle, dairy cattle, swine, and mammalian wildlife on Southern Ontario farms	**M. Viswanathan**[1], D.L. Pearl[2], E.N. Taboada[3], E.J. Parmley[4], C.M. Jardine[5]; [1]University of Guelph, Guelph, ON, Canada, [2]Department of Population Medicine, University of Guelph, Guelph, ON, Canada, [3]Laboratory for Foodborne Zoonoses, Public Health Agency of Canada, Lethbridge, AB, Canada,[4]Public Health Agency of Canada, Guelph, ON, Canada, [5]Department of Pathobiology, University of Guelph, Guelph, ON, Canada.
11:00	81	Isolation and characterization of Salmonella spp. from captive wild animals	**V.M. Belamaranahally**[1], N. Jaisingh[1], R. Doddamane[1], N.M. Bhat[2], V. Mudalagiri[3], R.C. Prasad[4]; [1]Microbiology, Veterinary College, BANGALORE, India, [2]Medicine, Veterinary College, BANGALORE, India, [3]IAH VB, BANGALORE, India, [4]KVAFSU, BANGALORE, India.
11:15	82	Following Salmonella Heidelberg through a poultry integrator.	**M. Madsen**[1], R. Sanchez-Inguanza[1], J. Guard[2], J. El-Attrache[1]; [1]Ceva Biomune, Lenexa, KS, USA, [2]Egg Safety and Quality Research Unit, USDA, ARS, Athens, GA, USA.

Epidemiology and Animal Health Economics Salon A/B/C/D - 5 th Floor Section Leader: Ashley Hill		
4:30		Break and Table Top Exhibits - Foyer
5:00	6:30	Poster Session II Grand Ballroom Salon III - 7th Floor

Immunology Salon F/G/H - 5th Floor Session Leader: Renukaradhya Gourapura			
TIME	**NO**	**TITLE**	**AUTHORS**
8:00	83	T lymphocytes induced after infection with a single PRRSV strain recognize epitopes processed from highly diverse PRRSV strains	G. Chung[1], A. Grimm[2], S. Cha[3], J. Rzepka[4], L. Karriker5[5], K.-J. Yoon[5], **C. Chung**[2]; [1]VMP, Washington State University, Pullman, WA, USA, [2]R&D, VMRD Inc., Pullman, WA, USA, [3]Virology Division, QIA, Anyang, Korea, Republic of, [4]VMRD Inc., Pullman, WA, USA, [5]Iowa State University, Ames, IA, USA.
8:15	84	Measuring bovine γδ T cell function at the site of Mycobacterium bovis infection	**R.A. Rusk**[1], M.V. Palmer[2], J.L. McGill[1], W.R. Waters[2]; [1]Diagnostic Medicine and Pathobiology, Kansas State University, Manhattan, KS, USA, [2]National Animal Disease Center, Ames, IA, USA.
8:30	85	Characterization of recombinant PRRSV nsp1beta mutants in a nursery pig model	**D. Shyu**[1], Y. Li[2], K. Ouyang[1], S. Dhakal[1], J. Hiremath[1], B. Binjawadagi[1], P. Shang[2], Y. Fang[2], R. Gourapura[1]; [1]Food Animal Health Research Program (FAHRP), Veterinary Preventive Medicine, The Ohio State University, OARDC, Wooster, OH, USA, [2]Diagnostic Medicine and Pathobiology, Kansas State Veterinary Diagnostic Laboratory, Kansas State University, College of Veterinary Medicine, Manhattan, KS, USA.
8:45	86	Reduced antigen-specific antibody levels in cows naturally infected with bovine leukemia virus	**M.C. Frie**[1], L.M. Sordillo[2], P.C. Bartlett[2], P.M. Coussens[3]; [1]Cell and Molecular Biology Program, Michigan State University, East Lansing, MI, USA, [2]Department of Large Animal Clinical Sciences, Michigan State University, East Lansing, MI, USA, [3]Department of Animal Science, Michigan State University, East Lansing, MI, USA.
9:00	87	Identification of immunodominant B cell epitopes in the C. pecorum proteome	**K. Rahman**, E. Chowdhury, Y.-C. Juan, B. Kaltenboeck; Pathobiology, Auburn University, Auburn, AL, USA.
9:15	88	A synthetic biodegradable microsphere vaccine of femtomole-dosed peptide antigens protects better against Chlamydia abortus than previous infection	**E.U. Chowdhury**, K. Rahman, B. Kaltenboeck; Pathobiology, Auburn University, Auburn, AL, USA.
9:30		**Break and Table Top Exhibits - Foyer**	
10:00	89	15-F2t-Isoprostane concentrations correlate with oxidant status in lactating dairy cattle with acute coliform mastitis.	**V. Mavangira**[1], M.J. Mangual[2], J.C. Gandy[1], L.M. Sordillo[1]; [1]Large Animal Clinical Sciences, Michigan State University, East Lansing, MI, USA, [2]Animal Science, Michigan State University, East Lansing, MI, USA.
10:15	90	Regulation of host immune gene expression by Torque Teno Sus Virus1 (TTSuV1) non-structural proteins	**P. Singh**, S. Ramamoorthy; Vet & Micro Sciences, North Dakota State University, Fargo, ND, USA.
10:30	91	Nsp1 and a part of Nsp2 genes of a synthetic porcine reproductive and respiratory syndrome virus are responsible for the viral capacity to induce type I interferons	**H. Sun**; School of Veterinary Medicine and Biomedical Sciences, University of Nebraska-Lincoln, Lincoln, NE, USA.

Immunology Salon F/G/H - 5th Floor Session Leader: Renukaradhya Gourapura			
10:45	92	Nanoparticle based Vaccination strategy against Swine Influenza Virus	**S. Dhakal**[1], J. Hiremath[1], J. Goodman[2], Y. SL[1], B. Shyu[1], K. Oyuang[1], K. Bondra[1], B. Binjawadagi[1], B. Narasimhan[2], C.W. Lee[1], R.J. Gourapura[1]; [1]Food Animal Health Research Program, Department of Veterinary Preventive Medicine, The Ohio State University, Wooster, OH, USA, [2]Department of Chemical and Biological Engineering, Iowa State University, Ames, IA, USA.
11:00	93	The impact of abomasal infusion of linoleic acid or linolenic acid on plasma fatty acid and oxylipid biosynthesis following Streptococcus uberis exposure	**V.E. Ryman**[1], B. Norby[1], N. Packiriswamy[1], S.E. Schmidt[2], A.L. Lock[2], L.M. Sordillo[1];1Large Animal Clinical Sciences, Michigan State University, East Lansing, MI, USA, 2Animal Science, Michigan State University, East Lansing, MI, USA.
11:15	94	Bovine gamma delta T cells and Th17 cells produce IL-17 in response to respiratory syncytial virus and Mannheimia haemolytica: implications for bovine respiratory disease	**J.L. McGill**[1], R.E. Briggs[2], R.E. Sacco[2]; [1]Diagnostic Medicine and Pathobiology, Kansas State University, Manhattan, KS, USA, [2]Ruminant Diseases and Immunology Research Unit, National Animal Disease Center, ARS, USDA, Ames, IA, USA.
11:30		**Lunch**	
1:00	95	First encounters: Mucosal immune system development and the microbiome	**P.J. Griebel**; School of Public Health and VIDO-Intervac, University of Saskatchewan, Saskatoon, SK, Canada.
1:45	96	Mucosal immunology: microbial interaction and cytokine production	**C.C.L. Chase**[1], C. Rinehart[2], K. Barling[3]; [1]Animal Disease Research & Diagnostic Lab., South Dakota State University, Brookings, SD, USA, [2]RTI, Brookings, SD, USA, [3]Lallemand Animal Health, Milwaukee, WI, USA.
2:15	97	An essential role of igt for pathogen clearance and microbiome homeostasis at mucosal surfaces of fish	**O. sunyer**[1], Z. Xu[2], F. Takizawa[2], D. Parra[2], D. Gomez[2], S. LaPatra[2], I. Salinas[2]; [1]Pathobiology, School of Veterinary Medicine, University of Pennsylvania, philadelphia, PA, USA, [2]Pathobiology, School of Veterinary Medicine, University of Pennsylvania, Philadelphia, PA, USA.
2:45		**Break and Table Top Exhibits - Foyer**	
3:00	98	Inflammatory mediator expression in lung epithelial cells and α/β T cells: roles in immunopathogenesis associated with respiratory syncytial virus infection in calves	**R.E. Sacco**; Ruminant Diseases and Immunology Research Unit, National Animal Disease Center/USDA/ARS, Ames, IA, USA.
3:30	99	PIV-3 blocks antiviral mediators downstream of the IFN-λR by modulating Stat1 phosphorylation	**K. Eberle**[1], J. McGill[2], T. Reinhardt[1], R. Sacco[1]; [1]National Animal Disease Center, Ames, IA, USA, [2]Kansas State University, Manhattan, KS, USA.
3:45	100	From Swine Dysentery to Inflammatory Bowel diseases: Role of the Resident Microbiota in Tuning the Host Response.	**M. Wannemuehler**; Vet Microbiology & Preventive Medicine, Iowa State University, Ames, IA, USA.

Immunology Salon F/G/H - 5th Floor Session Leader: Renukaradhya Gourapura			
4:15	101	PEDV shedding patterns and antibody kinetics in commercial growing pigs	**J.B. Kraft**[1], K. Woodard[1], L. Gimenez-Lirola[1], M. Rotolo[1], C. Wang[2], P. Lasley[3], Q. Chen[1], J.Q. Zhang[1], D. Baum[1], P. Gauger[1], K.J. Yoon[1], J. Zimmerman[1], R. Main[1]; [1]Veterinary Diagnostic Laboratory, Iowa State University, Ames, IA, USA, [2]Department of Statistics, Iowa State University, Ames, IA, USA, [3]Murphy Brown of Missouri, Princeton, MO, USA.
4:30		**Break and Table Top Exhibits - Foyer**	
5:00	**6:30**	**Poster Session II Grand Ballroom Salon III - 7th Floor**	
TUES. 8:00:00 AM	102	Cellular response following Digital Dermatitis infection	**J. Wilson-Welder**, J. Nally, S. Humphrey, D. Alt; Infectious Bacterial Disease of Livestock, National Animal Disease Center, ARS-USDA, Ames, IA, USA.
8:15	103	Montanide™ adjuvant technologies for influenza vaccines.	**S. Xu**[1], J. Ben Arous[2], J. Gaucheron[2], L. Dupuis[2]; [1]20 Two Bridges Road, Seppic Inc, Fairfield, NJ, USA, [2]SEPPIC, Puteaux Cedex, France.
8:30	104	Expression of interferon-beta (IFN-β) by dendritic cells activated with Streptococcus suis	**J.-P. Auger**, A. Santinon, P. Lemire, M. Segura, M. Gottschalk; GREMIP & CRIPA, Faculty of Veterinary Medicine, University of Montreal, Saint-Hyacinthe, QC, Canada.
8:45	105	Intramammary 25-hydroxyvitamin D3 treatment increases vitamin D pathway activity but not acute host-defense responses to endotoxin-induced mastitis.	K.E. Merriman, J.L. Powell, J.E.P. Santos, **C.D. Nelson**; Department of Animal Sciences, University of Florida, Gainesville, FL, USA.
9:00	106	A novel vector platform for vaccine delivery in domestic animal species	M. Martins[1], L.R. Joshi[2], K.S. Hain[2], D. Anziliero[3], R. Frandoloso[4], D.L. Rock[5], R. Weiblen[1], E.F. Flores[1], **D.G. Diel**[2]; [1]Preventive Veterinary Medicine, Federal University of Santa Maria (UFSM), Santa Maria, Brazil, [2]Veterinary and Biomedical Sciences, South Dakota State University, Brookings, SD, USA, [3]Faculdade de Medicina, Faculdade Meridional - IMED, Passo Fundo, Brazil, [4]Faculdade de Agronomia e Medicina Veterinária, Universidade de Passo Fundo, Passo Fundo, Brazil, [5]Pathogiology, University of Illinois at Urbana-Champaign, Urbana, IL, USA.
9:15	107	Porcine reproductive and respiratory syndrome virus non-structural protein Nsp2TF down-modulates Swine Leukocyte Antigen class I (SLA class I) expression	**Q. Cao**, S. Subramaniam, Y. Ni, D. Cao, X. Meng; Biomedical Science and Pathobiology, Virginia Tech, Blacksburg, VA, USA.
9:30AM		**Break**	

Immunology Salon F/G/H - 5th Floor Session Leader: Renukaradhya Gourapura			
10:00	108	Endonuclease G participates in caspase-independent apoptosis induced by Mycobacterium bovis in bovine macrophages.	A. Benítez-Guzmán[1], L. Arriaga-Pizano[2], J. Morán[3], **J.A. Gutierrez-Pabello**[1]; [1]Laboratorio de Investigación en Tuberculosis y Brucelosis, Facultad de Medicina Veterinaria y Zootecnia, Universidad Nacional Autónoma de México, Mexico City, Mexico, [2]Unidad Médica de Investigación en Inmunoquímica, Hospital Siglo XXI, Mexico City, Mexico, [3]Instituto de Fisiología Celular, Universidad Nacional Autónoma de México, Mexico City, Mexico.
10:15	109	Pathogenesis comparison of the U.S. PEDV prototype and S-INDEL-variant strains in weaned pigs and examination of the cross-protective immunity of two virus strains	**Q. Chen**, M. Stafne, M. Spadaro, H. Salzbrenner, P. Gauger, M. Welch, J. Thomas, P. Arruda, L. Gimenez-Lirola, D. Magstadt, M. Darin, J. Zhang; VDPAM, Iowa State University, Ames, IA, USA.
10:30	110	Genome wide association study identifies loci associated with somatic cell count phenotypes following experimental challenge with Streptococcus uberis	L.J. Siebert[1], M.E. Staton[2], S.P. Oliver[3], **G.M. Pighetti**[1]; [1]Department of Animal Science, University of Tennessee, Knoxville, TN, USA, [2]Department of Entomology and Plant Pathology, University of Tennessee, Knoxville, TN, USA, [3]AgResearch, University of Tennessee, Knoxville, TN, USA.
10:45	111	Bordetella bronchiseptica colonization has minimal impact on live-attenuated influenza virus vaccine cross-protective efficacy in pigs.	**C.L. Loving**, H.R. Hughes, S.L. Brockmeier; USDA-ARS-National Animal Disease Center, Ames, IA, USA.
11:45	**12:30**	**Business Meeting, Dedication and Graduate Student Competition Awards Presentations**	

Pathobiology of Enteric and Foodborne Pathogens
Michigan/Michigan State - 6th Floor
Section Leader Weiping Zhang

TIME	NO	TITLE	AUTHORS
8:15	112	Characterization and application of monoclonal antibodies against porcine epidemic diarrhea virus	**Y. Wang**[1], R. Guo[1], R. Ransburgh[2], J. Hill[2], J. Henningson[1], W. Zhang[1], Y. Fang[1]; [1]Diagnostic Medicine/ Pathobiology, College of Veterinary Medicine, Kansas State University, Manhattan, KS, USA, [2]Kansas State Veterinary Diagnostic Laboratory, College of Veterinary Medicine, Kansas State University, Manhattan, KS, USA.
8:30	113	Pathogenicity and physicochemical properties of Salmonella Typhimurium treated with natural phenolics from industry byproducts	**S. Salaheen**, E. Jaiswal, M. Peng, D. Biswas; Department of Animal and Avian Sciences, University of Maryland-College Park, College Park, MD, USA.
8:45	114	Enhancing food safety for human consumers by eliminating food-borne enteric pathogens	**R. Curtiss, III**; College of Veterinary Medicine, University of Flordia, Gainsville, FL, USA.
9:30		**Break and Table Top Exhibits - Foyer**	
10:00	115	Salmonella Pathogenicity Island 13 contributes to pathogenesis in streptomycin pre-treated mice but not in day-old chickens.	**J.R. Elder**[1], K. Chiok[1], N.C. Paul[1], G. Haldorson[1], J. Guard[2], D.H. Shah[1]; [1]Department of Veterinary Microbiology and Pathology, Washington State University, Pullman, WA, USA, [2]Egg Quality and Safety Research Unit, United States Department of Agriculture, Athens, GA, USA.
10:15	116	Salmonella pathogenicity island 13 contributes to the metabolic fitness of Salmonella Enteritidis through glucuronic acid and tyramine metabolism.	**J.R. Elder**, J. Guard, D.H. Shah; Department of Veterinary Microbiology and Pathology, Washington State University, Pullman, WA, USA.
10:30	117	Quantification of coliforms and Escherichia coli on beef carcasses immediately before and after evisceration during slaughter.	**L.W. Noll**[1], P.B. Shridhar[1], S.D. Menon[1], X. Shi[1], S.E. Ives[2], T.E. Lawrence[2], E. Cha[1], T. Nagaraja[1], D.G. Renter[1]; [1]Diagnostic Medicine and Pathobiology, Kansas State University, Manhattan, KS, USA, [2]Agricultural Sciences, West Texas A&M University, Canyon, TX, USA.
10:45	118	Quantification of microbial transfer from hides to carcasses in commercial beef slaughter operations	**A. McKiearnan**, N. Cernicchiaro, M. Sanderson; Diagnostic Medicine/Pathobiology, Kansas State University, Manhattan, KS, USA.
11:00	119	Bayesian estimation of sensitivity and specificity of culture- and PCR-based methods for the detection of the six major non-O157 Escherichia coli serogroups in cattle feces	**P.S. Ekong**, M.W. Sanderson, P.B. Shridhar, N. Cernicchiaro, D.G. Renter, J. Bai, T.G. Nagaraja; Department of Diagnostic Medicine / Pathobiology, Kansas State University, Manhattan, KS, USA.

Pathobiology of Enteric and Foodborne Pathogens Michigan/Michigan State - 6th Floor Section Leader Weiping Zhang			
11:15	120	Associations between diet, gut microbiome, and health in red-shanked doucs (Pygathrix nemaeus): a model for captive primate health	**J.B. Clayton**[1], D. Knights[2], H. Huang[3], L.T. Ha[4], T.V. Bui[4], M.V. Vo[5], D.A. Travis[6], F. Cabana[7], T.J. Johnson[1]; [1]Dept. of Veterinary and Biomedical Sciences, University of Minnesota, Saint Paul, MN, USA, [2]Dept. of Computer Science and Engineering, University of Minnesota, Minneapolis, MN, USA,[3]Dept. of Biomedical Informatics and Computational Biology, University of Minnesota, Minneapolis, MN, USA, [4]GreenViet Biodiversity Conservation Center, Danang, Viet Nam, [5]Danang University of Education, Danang, Viet Nam, [6]Dept. of Veterinary Population Medicine, University of Minnesota, Saint Paul, MN, USA, [7]Dept. of Social Sciences, Oxford Brookes University, Oxford, UK.
11:30		**Lunch**	
1:15	121	Lactobacillus reuteri derived-histamine suppress interleukin-6 by inhibiting H1-receptor downstream signaling in germ-free mice	**B. Ganesh**, J. Versalovic; Pathology and Immunology, Baylor College of Medicine, Houston, TX, USA.
1:45	122	Host defense peptide-inducing compounds as alternatives to antibiotics	**G. Zhang**; Department of Animal Science, Oklahoma State University, Stillwater, OK, USA.
2:15	123	From single probiotics to complex commensal microbiota: effects on immunity, enteric infections and vaccines in gnotobiotic pigs.	**A.N. Vlasova**[1], S. Kandasamy[1], L. Shao[1], D.D. Fischer[1], A. Rauf[1], S.N. Langel[1], K.S. Chattha[2], A. Kumar[1], H.-C. Huang[1], M. Abdulhameed[1], G. Rajashekara[1], L.J. Saif[1]; [1]FAHRP/OARDC, The Ohio State University, Wooster, OH, USA, [2]Canadian Food Inspection Agency, Lethbridge, AB, Canada.
2:45		**Break and Table Top Exhibits - Foyer**	
3:00	124	Microbial shifts in the swine distal gut caused by the antimicrobial growth promoter tylosin	**R. Isaacson**[1], H.B. Kim[2]; [1]Veterinary and Biomedical Sciences, University of Minnesota, St. Paul, MN, USA, [2]Department of Animal Resources, Dankook University, Cheonan, Korea, Republic of.
3:30	125	The hunt for alternatives to antibiotics in poultry: a systematic, microbial community-based approach	**T.J. Johnson**[1], S.L. Noll[2], B.P. Youmans[1], K.A. Case[1], J.L. Danzeisen[1], J.A. Brannon[2]; [1]Veterinary and Biomedical Sciences, University of Minnesota, Saint Paul, MN, USA, [2]Animal Science, University of Minnesota, Saint Paul, MN, USA.
4:00	126	Commensal gut bacteria as new generation probiotics to improve gut health	**J. Scaria**; Veterinary and Biomedical Science, South Dakota State University, Brookings, SD, USA.
4:30	127	The swine intestinal microbiota: localized responses to in-feed antibiotics	**T. Looft**, H.K. Allen; FSEP, USDA-ARS-NADC, Ames, IA, USA.
4:30		**Break and Table Top Exhibits - Foyer**	
5:00	**6:30**	**Poster Session II Grand Ballroom Salon - 7th Floor**	

Pathobiology of Enteric and Foodborne Pathogens
Michigan/Michigan State - 6th Floor
Section Leader Weiping Zhang

Time	NO	TITLE	AUTHORS
Tues. 8:15	128	A highly-sensitive, field-deployable molecular assay for rapid detection of porcine epidemic diarrhea virus	**A. Ambagala**[1], M. Fisher[2], J. Pasick[3], O. Lung[1], K.J. Yoon[4], J. Keenliside[5], C. Buchanan[1], N. Thanthrige-Don[2], T. Furukawa-Stoffer[1], P.A. Lee[6], S. Alexandersen[7]; [1]National Centres for Aniimal Disease, Canadian Food Inspection Agency, Lethbridge, AB, Canada, [2]Faculty of Veterianry Medicine, University of Calgary, Calgary, AB, Canada, [3]National Centres for Aniimal Disease, Canadian Food Inspection Agency, Winnipeg, MB, Canada, [4]College of Veterinary Medicine, Iowa State University, Ames, IA, USA, [5]Alberta Agriculture and Forestry, Edmonton, AB, Canada, [6]GeneReach USA, Lexington, MA, USA, [7]Formerly at the National Centres for Aniimal Disease, Canadian Food Inspection Agency, Winnipeg, MB, Canada.
8:30	129	Bayesian estimation of sensitivity and specificity of culture- and PCR-based methods for the detection of Escherichia coli O157 in cattle feces	**P.S. Ekong**, M.W. Sanderson, L.W. Noll, N. Cernicchiaro, D.G. Renter, J. Bai, T.G. Nagaraja; Department of Diagnostic Medicine / Pathobiology, Kansas State University, Manhattan, KS, USA.
8:45	130	Optimizing & standardizing anti-STa antibody titration assay by using ovalbumin-STa fusion protein as ELISA coating antigen	**Q. duan**, Z. chen, W. zhang; Diagnostic Medicine/Pathobiology, Kansas State Univerisity College of Veterinary Medicine, Manhattan, KS, USA.
9:00	131	A multiepitope fusion antigen of fimbrial adhesin tips of enterotoxigenic Escherichia coli (ETEC) indices broadly protective anti-adhesin antibodies	**R.M. Nandre**[1], X. Ruan[1], D. Sack[2], W. Zhang[1]; [1]Diagnostic Medicine and Pathobiology, College of Veterinary Medicine, Kansas State University, Manhattan, KS, USA, [2]International Health,, Johns Hopkins University Bloomberg School of Public Health,, Baltimore,, MD, USA.
9:15	132	Adjuvanticity of double mutant heat-labile toxin (dmLT, LTR192G/L211A) of enterotoxigenic Escherichia coli (ETEC) in mouse parenteral immunizations with a toxoid fusion antigen	**R.M. Nandre**, X. Ruan, W. Zhang; Diagnostic Medicine and Pathobiology, College of Veterinary Medicine, Kansas State University, Manhattan, KS, USA.
9:30		**Break**	
10:00	133	Effect of ascorbic acid on survival and bacterial contents in the gut contents of oreochromis niloticus	**F. Rasool**, S. Parveen, N. Atta; Fisheries and Aquaculture, University of Veterinary and Animal Sciences, Lahore-Pakistan, Lahore, Pakistan.
10:15	134	Identification of potential probiotic species for growth promotion in turkey flocks.	**B.P. Youmans**[1], K.A. Case[1], S.L. Noll[2], B. Lindquist[3], J.L. Danzeisen[1], T.J. Johnson[1]; [1]Veterinary and Biomedical Sciences, University of Minnesota, St Paul, MN, USA, [2]Animal Science, University of Minnesota, St Paul, MN, USA, [3]Phileo-Lesaffre Animal Care, Humboldt, IA, USA.
11:45	**12:30**	**Business Meeting, Dedication, and Graduate Student Competition Award Presentation**	

Respiratory Diseases Indiana/Iowa Room 6th Floor Section Leader: Amelia Woolums			
TIME	**NO**	**TITLE**	**AUTHORS**
MON. 8:15:00 AM	135	ORF5 sequencing indicated PRRS strain shifting in the field	J. Bai, S. Henry, E. Poulsen, L. Tokach, M. Potter, D. Hesse, G. Anderson; Kansas State Veterinary Diagnostic Laboratory, Kansas State University, Manhattan, KS, USA, Abilene Animal Hospital, Abilene, KS, USA.
8:30	136	Detection of Actinobacillus pleuropneumoniae ApxIV toxin antibody in serum and oral fluid specimens from pigs inoculated with under experimental conditions.	**W. Gonzalez**[1], L. Giménez-Lirola[1], M. Gottschalk[2], A. Holmes[1], S. Lizano[3], C. Goodell[3], K. Poonsuk[1], J. Zimmerman[1], P. Sitthicharoenchai[1], C. Wang[1]; [1]Iowa State University, Ames, IA, USA, [2]Université de Montréal, St. Hyacinthe, QC, Canada, [3]IDEXX Laboratories, Inc, Westbrook, ME, USA.
8:45	137	Genetic diversity of porcine reproductive and respiratory syndrome virus genes determined by metagenomic sequencing of clinical samples	**E. Collin**[1], E. Schirtzinger[1], L. Peddireddi[1], P. Gauger[2], T. Clement[3], B. Hause[1]; [1]Veterinary Diagnostic Lab, Kansas State University, Manhattan, KS, USA, [2]Iowa State University, Ames, IA, USA, [3]South Dakota State University, Brookings, SD, USA.
9:00	138	Discovery and pathogenesis of porcine parainfluenza-1 in pigs in the United States	**R. Palinski**[1], Z. Chen[1], J.N. Henningson[2], R.R.R. Rowland[1], Y. Fang[1], B.M. Hause[1]; [1]Department of Diagnostic Medicine and Pathobiology, Kansas State University, Manhattan, KS, USA, [2]Kansas State Veterinary Diagnostic Laboratory, Kansas State University, Manhattan, KS, USA.
9:15	139	Evaluation of the use of non-pathogenic porcine circovirus type 1 as a vaccine delivery virus vector to express antgenic determinants of porcine reproductive and respiratory syndrome virus	**P.E. Pineyro**[1], S. Kenney[1], L.G. Giménez-Lirola[2], T. Opriessnig[3], D. Tian[1], C.L. Heffron[1], X.J. Meng[1]; [1]Virginia Tech, Blacksburg, VA, USA, [2]Iowa State University, Ames, IA, USA, [3]The Royal (Dick) School of Veterinary Studies, Edinburgh, UK.
9:30		**Break and Table Top Exhibits - Foyer**	
10:00	140	Agreement among sampling methods used to identify viral and bacterial pathogens in dairy calves with bovine respiratory disease (BRD)	**A. Woolums**[1], D. Doyle[1], R. Berghaus[2], B. Credille[2], T. Lehenbauer[3], S. Aly[3], J. Champagne[3], P. Blanchard[3], B. Crossley[3]; [1]Department of Pathobiology and Population Medicine, Mississippi State University, Mississippi State, MS, USA, [2]Department of Population Health, University of Georgia, Athens, GA, USA, [3]Department of Population Health and Reproduction, University of California, Davis, Davis, CA, USA.
10:15	141	Iodine secretion in airway surface fluid following a single oral bolus of sodium iodide in calves.	**B.M. Shoemake**[1], B.L. Vander Ley[1], M.A. Klingenberg[1], R.A. Nolan[1], A.M. Meyer[2], L.G. Schultz[1], B.W. Newcomer[3], M.C. Heller[4]; [1]College of Veterinary Medicine, University of Missouri, Columbia, MO, USA, [2]Department of Animal Sciences, College of Agriculture, Food and Natural Resources, University of Missouri, Columbia, MO, USA, [3]Department of Pathobiology, College of Veterinary Medicine, Auburn University, Auburn, AL, USA, [4]Department of Medicine and Epidemiology, School of Veterinary Medicine, University of California, Davis, Davis, CA, USA.

Respiratory Diseases Indiana/Iowa Room 6th Floor Section Leader: Amelia Woolums			
10:30	142	In vitro inactivation of bovine viral respiratory pathogens using an iodine-based antimicrobial system	**B. Newcomer**[1], B. Vander Ley[2], P. Galik[1], M. Heller[3]; [1]Department of Pathobiology, Auburn University, Auburn, AL, USA, [2]University of Missouri College of Veterinary Medicine, Columbia, MO, USA, [3]University of California Davis School of Veterinary Medicine, Davis, CA, USA.
10:45	143	Sodium iodide inactivates Rhodococcus Equi in vitro	**M.C. Heller**[1], F. Smith[2], K. Jackson[1], J.L. Watson[1]; [1]Veterinary Medicine and Epidemiology, University of California Davis, Davis, CA, USA, [2]Veterinary Medical Teaching Hospital, University of California Davis, Davis, CA, USA.
11:00	144	Sodium iodide inactivates Manheimia hemolytica and Bibersteinia trehalosi in vitro.	**M.C. Heller**[1], K.A. Clothier[2], B.W. Newcomer[3], B.L. VanderLey[4]; [1]Veterinary Medicine and Epidemiology, University of California Davis, Davis, CA, USA, [2]California Animal Health and Food Safety Laboratory, University of California Davis, Davis, CA, USA, [3]Department of Pathobiology, Auburn University, Auburn, AL, USA, [4]Veterinary Medicine and Surgery, University of Missouri, Columbia, MO, USA.
11:15	145	Zelnate™: a novel approach to BRD management in cattle	**A.J.S. Abraham**, J. Nickell, D. Keil, T. Settje; R&D, Bayer Animal Health, Shawnee, KS, USA.
11:30		**Lunch**	
1:30	146	Evaluation of response to vaccination on the feedlot performance of weaned calves.	**J.J. Gaspers**, G.L. Stokka, K.C. Swanson; Animal Science, North Dakota State University, Fargo, ND, USA.
1:45	147	Evaluation of on-arrival vaccination and deworming on stocker cattle health and growth performance	**C.M. Griffin**[1], B. Karisch[2], A.R. Woolums[1], J. Blanton[2], R.M. Kaplan[3], W. Epperson[1], D.R. Smith[1]; [1]Mississippi State University College of Veterinary Medicine, Mississippi State, MS, USA, [2]Animal and Dairy Sciences, Mississippi State University, Mississippi State, MS, USA, [3]University of Georgia College of Veterinary Medicine, Athens, GA, USA.
2:00	148	Comparison of the immune response to subcutaneous or intranasal modified-live virus booster vaccination in young beef calves that were primed with intranasal vaccine	**J.H. J. Bittar**[1], T.A. Collins[2], D.J. Hurley[1], A.R. Woolums[3], R.A. Palomares[1]; [1]Department of Population Health. College of Veterinary Medicine, University of Georgia, Athens, GA, USA, [2]College of Veterinary Medicine, Tuskegee University, Tuskegee, AL, USA, [3]Department of Pathobiology and Population Medicine. College of Medicine Veterinary, Mississippi State University, Mississippi State, MS, USA.
2:15	149	Immune responses, clinical and pathological outcomes in challenged calves immunized with a subunit vaccine for BRSV and H.somni	**L.J. Gershwin**[1], F.R. Carvallo[1], H.A. McEligot[1], N.E. Behrens[1], L.T. Crum[2], B.M. Gunnarson[1], L.B. Corbeil[2]; [1]School of Veterinary Medicine, Dept. of Pathology, Microbiology, & Immunology, University of California, Davis, Davis, CA, USA, [2]School of Medicine, Dept of Pathology, University of California, San Diego, San Diego, CA, USA.

Respiratory Diseases Indiana/Iowa Room 6th Floor Section Leader: Amelia Woolums			
2:30	150	Histophilus somni increases expression of antiviral proteins in bovine respiratory epithelial cells.	C. Lin[1], J.T. Agnes[1], N. Behrens[2], M. Shao[2], Y. Tagawa[3], L.J. Gershwin[2], **L.B. Corbeil**[1]; [1]Pathology, UCSD, San Diego, CA, USA, [2]Pathology, Microbiology, Immunology, UC Davis, Davis, CA, USA, [3]National Institute of Animal Health, Tsukuba, Japan.
2:45		**Break and Table Top Exhibits - Foyer**	
3:00	151	Inhibition of Pasteurella multocida biofilm formation by capsular polysaccharide, and interaction with Histophilus somni in a polymicrobial biofilm	B. Petruzzi[1], W.K. Scarratt[2], V. Buechner-Maxwell[1], **T.J. Inzana**[1]; [1]Biomedical Sciences and Pathobiology, Virginia Tech, Blacksburg, VA, USA, [2]Large Animal Clinical Sciences, Virginia Tech, Blacksburg, VA, USA.
3:15	152	Genetic engineering of cattle that produce leukocytes resistant to Mannheimia haemolytica leukotoxin	**S. Shanthalingam**[1], P. Kasinathan[2], J.E. Beever[3], A. Tibary[4], W.C. Brown[1], S. Srikumaran[1]; [1]Department of Veterinary Microbiology and Patholog, Washington State Universtiy, Pullman, WA, USA, [2]Trans Ova Genetics, Sioux Center, IA, USA, [3]University of Illinois, Urbana, IL, USA, [4]Veterinary Clinical Sciences, Washington State Universtiy, Pullman, WA, USA.
3:30	153	Mannheimia haemolytica leukotoxin is cytotoxic even in the absence of acylation.	**S.A. Batra**[1], S. Shanthalingam[1], G.R. Munske[2], B. Raghavan[1], A. Kugadas[1], J. Bavanthasivam[1], S.K. Highlander[3]; [1]Department of Veterinary Microbiology and Pathology, Washington State University, Pullman, WA, USA, [2]Molecular Biology and Genomics Core, Washington State University, Pullman, WA, USA, [3]J. Craig Venter Institute, La Jolla, CA, USA.
3:45	154	Development of a nucleotide polymorphism-based typing method for Mannheimia haemolytica and identification of a subtype that associates with bovine respiratory disease	**M. Clawson**; USDA/ARS/ US Meat Animal Research Center, Clay Center, NE, USA.
4:30	**5:00**	**Break and Table Top Exhibits - Foyer**	
5:00	**6:30**	**Poster Session II Grand Ballroom Salon II - 7th Floor**	

Vector - Borne And Parasitic Disesases Denver/Houston - 5th Floor Section Leader: Roman Ganta			
TIME	**NO**	**TITLE**	**AUTHORS**
MON 8:00	155	Vaccination with an attenuated mutant of Ehrlichia chaffeensis induces pathogen-specific CD4 T cell immunity and protection from wildtype challenge in a canine host	**J.L. McGill**[1], A.D.S. Nair[1], C. Cheng[1], R.A. Rusk[2], D.C. Jaworski[1], R.R. Ganta[1]; [1]Center of Excellence for Vector-Borne Diseases, Diagnostic Medicine and Pathobiology, Kansas State University, Manhattan, KS, USA, [2]Pathobiology Graduate Program, Diagnostic Medicine and Pathobiology, Kansas State University, Manhattan, KS, USA.
8:15	156	Development and validation of real-time PCR assay for canine Lyme disease	**A. DS Nair**[1], L. Peddireddi[2], J. Bai[2], G. Anderson[2], R.R. Ganta[1]; [1]Diagnostic Medicine and Pathobiology, College of Veterinary Medicine, Kansas State University, Manhattan, KS, USA, [2]Kansas State Veterinary Diagnostic Laboratory, Kansas State University, Manhattan, KS, USA.
8:30	157	Comparison of an alternative diagnostic sampling technique for Tritrichomonas foetus in cattle	**G.A. Dewell**, K.M. Harmon, T.M. Dohlman, P.E. Phillips, P.C. Gauger; VDPAM, Iowa State University, Ames, IA, USA.
8:45	158	Development of a recombinant subunit vaccine for Rift Valley fever	**B. Faburay**[1], N.N. Gaudreault[1], V. Shivanna[1], S. Sunwoo[1], W. Ma[1], I. Morozov[1], A.S. Davis[1], M. Ruder[2], B. Drolet[3], D.S. McVey[4], W.C. Wilson[2], J.A. Richt[1]; [1]Diagnostic Medicine/Pathobiology, Kansas State University, Manhattan, KS, USA, [2]Arthropod Borne Animal Disease Research Unit, United States Department of Agriculture, Agricultural Research Service, Manhattan, KS, USA, [3]Arthropod Borne Animal Disease Research Unit, Uinted States Department of Agriculture, Agricultural Research Service, Manhattan, KS, USA, [4]Arthropod Borne Animal Disease Research Unit, United State Department of Agriculture, Agricultural Research Service, Manhattan, KS, USA.
9:00	159	Fish mucus; a physical barrier to pathogens	**M.A. Ashraf**; Fisheries and Wildlife, University of Veterinary and Animal Sciences Lahore, Pakistan, Lahore, Pakistan.
9:15	160	Development of a generic Ehrlichia FRET-qPCR and investigation of ehrlichioses in domestic ruminants on five Caribbean islands	**J. Zhang**[1], P. Kelly[2], W. Guo[3], C. Xu[1], L. Wei[1], F. Jongejan[4], A. Loftis[2], C. Wang[1]; [1]College of Veterinary Medicine, Yangzhou University, Yangzhou, China, [2]School of Veterinary Medicine, Ross University, Basseterre, NJ, USA, [3]College of Animal Science, Anhui Science and Technology University, Fengyang, China, [4]Faculty of Veterinary Medicine, Utrecht Centre for Tick-borne Diseases, Utrecht, Netherlands.
9:30		**Break and Table Top Exhibits - Foyer**	
10:00	161	Molecular approaches in understanding Ehrlichia pathogenesis, host-pathogen interactions and in developing vaccines	**R. Ganta**; Center of Excellence for Vector-Borne Diseases, Department of Diagnostic Medicine/Pathobiology, Kansas State University, Manhattan, OH, USA.
10:45	162	Sequence determinants spanning -35 motif and AT-rich spacer sequences impacting ehrlichia chaffeensis sigma 70-dependent promoter activity of two differentially expressed p28 outer membrane protein genes	H. Liu, **L. Jakkula**, T. Von-Ohlen, R. Ganta; Diagnostic Medicine/Pathobiology, College of Veterinary Medicine, Kansas State University, Manhattan, KS, USA.

Vector - Borne And Parasitic Disesases Denver/Houston - 5th Floor Section Leader: Roman Ganta			
TIME	**NO**	**TITLE**	**AUTHORS**
11:00	163	Cerebral nematodiasis in camelids: a retrospective study	**S.D. Taylor**[1], F.-R. Bertin[2]; [1]Veterinary Clinical Sciences, Purdue University, West Lafayette, IN, USA, [2]Physiology, McGill University, Montreal, QC, Canada.
11:15	164	Molecular detection of vector-borne agents in dogs from ten provinces of China	**W. Guo**[1], D. Xu[1], J. Zhang[1], S. Bu[1], Z. Shi[2], C. Song[3], X. Zheng[4], Y. Zhang[5], Y. Hao[6], H. Dong[7], L. Wei[1], H.S. El-Mahallawy[8], W. Xiong[1], H. Wang[1], J. Li[1], X. Zhang[1], P. Kelly[9], C. Wang[1]; [1]College of Veterinary Medicine, Yangzhou University, Yangzhou, China, [2]College of Veterinary Medicine, China Agricultural University, Beijing, China, [3]College of Animal Science & Technology, Yunnan Agricultural University, Kunming, China, [4]Jiangsu Agri-animal Husbandry Vocational College, Taizhou, China, [5]College of Veterinary Medicine, Xinjiang Agricultural University, Urumchi, China, [6]College of Veterinary Medicine, Inner Mongolia Agricultural University, Hohhot, China, [7]College of Animal Science and Veterinary Medicine, Henan Agricultural University, Zhengzhou, China, [8]Faculty of Veterinary Medicine, Suez Canal University, Ismailia, Egypt, [9]School of Veterinary Medicine, Ross University, Basseterre, NJ, USA.
11:30		**Lunch**	
1:30	165	Defining the long-term duration of parasitemia and antibody response in cattle infected with various strains and doses of babesia bovis and evaluating sero-diagnostic tools	**C. Chung**[1], C. Suarez[2], C. Wilson[1], C. Bandaranayaka-Mudiyanselage[1], J. Rzepka[3], A. Grimm[1], G. Chung[1], S. Lee[4]; [1]R&D, VMRD Inc., Pullman, WA, USA, [2]USDA-ADRU, Pullman, WA, USA, [3]VMRD Inc., Pullman, WA, USA, [4]University of Idaho, Moscow, ID, USA.
1:45	166	The epidemiological status of African swine fever in domestic swine herds in the Tavush Marz region, Republic of Armenia.	T. Markosyan, K. Sargsyan, S. Kharatyan, H. Elbakyan, V. Hakobyan, L. Simonyan, H. Voskanyan, A. Shirvanyan, T. Stepanyan, M. Khachatryan, R. Weller, G. Risatti; Scientific Centre for Risk Assessment and Analysis in Food Safety Area, SNCO, Yerevan, Armenia, Ministry of Agriculture of the Republic of Armenia, Yerevan, Armenia, Fundamental Computational and Sciences Division, Pacific Northwest National Laboratory, Richland, WA, USA, Pathobiology and Veterinary Science, College of Agriculture, Health and Natural Resources, University of Connecticut, Storrs, CT, USA.
4:30	**5:00**	**Break and Table Top Exhibits - Foyer**	
5:00	**6:30**	**Poster Session II Grand Ballroom Salon III - 7th Floor**	

Viral Pathogenesis Los Angeles/Miami Rooms - 5th Floor Section Leader: Kyoung-Jin Yoon			
TIME	**NO**	**TITLE**	**AUTHORS**
MON 8:15	167	Severe Fever with Thrombocytopenia Syndrome virus noncoding regions of S, M and L segments regulate RNA synthesis	**R. Pudupakam**, Y. Choi, J. Jung; Department of Molecular Microbiology and Immunology, University of Southern California, Los Angeles, CA, USA.
8:30	168	Characterization of the humoral immune responses to porcine epidemic diarrhea Virus (PEDV) infection in weaned pigs	**M. Bhandari**[1], H. Hoang[1], D. Sun[1], L.G. Giménez-Lirola[2], K. Shi[3], D.M. Madson[2], L. Labios[2], D. Magstadt[2], P.H.E. Arruda[2], Y. Fang[4], D. Yoo[3], K.-J. Yoon[1]; [1]Vet Micro and Prventiv Med, Iowa State University, Ames, IA, USA, [2]VDPAM, Iowa State University, Ames, IA, USA, [3]Department of Pathobiology, University of Illinois at Urbana-Champaign, Urbana, IL, USA, [4]Department of Diagnostic Medicine/Pathobiology, Kansas State University, Manhattan, KS, USA.
8:45	169	Porcine reproductive and respiratory syndrome virus hijacks nanotubes for intercellular spread: an alternative pathway used for nidovirus transmission	**R. Guo**[1], B.B. Katz[2], J. Tomich[2], Y. Fang[1]; [1]Diagnostic Medicine/Pathobiology, College of Veterinary Medicine, Kansas state university, Manhattan, KS, USA, [2]Department of Biochemistry and Molecular Biophysics, College of Arts and Sciences, Kansas state university, Manhattan, KS, USA.
9:00	170	Mutations in a highly conserved motif of nsp1beta protein attenuate the innate immune suppression function of porcine reproductive and respiratory syndrome virus (PRRSV)	**Y. Li**[1], D.-L. Shyu[2], P. Shang[1], J. Bai[1], K. Ouyang[2], S. Dhakal[2], J. Hireamt[2], B. Binjawadagi[2], G.J. Renukaradhya[2], Y. Fang[1]; [1]Diagnostic Medicine / Pathobiology, Kansas State university, Manhattan, KS, USA, [2]Food Animal Health Research Program (FAHRP), The Ohio State University, Wooster, OH, USA.
9:15	171	Attenuation of porcine reproductive and respiratory syndrome virus (PRRSV) by inactivating the ribosomal frameshifting products nsp2TF and nsp2N: Implication for the rational design of vaccines	**P. Shang**[1], Y. Li[1], D.-L. Shyu[2], C. Carrillo[3], G. Renukaradhya[2], J. Henningson[1], Y. Fang[1]; [1]Department of Diagnostic Medicine and Pathobiology, Kansas state university, Manhattan, KS, USA, [2]Food Animal Health Research Program (FAHRP), Veterinary Preventive Medicine, The Ohio State University, Wooster, OH, USA, [3]Physical & Life Sciences Directorate, Lawrence Livermore National Laboratory, Livermore, CA, USA.
9:30		**Break and Table Top Exhibits - Foyer**	
10:00	172	Effects of adenoviral delivered interferon-alpha on porcine reproductive and respiratory syndrome virus infection in swine	**S.L. Brockmeier**, C.L. Loving; Virus and Prion Research Unit, National Animal Disease Center, Ames, IA, USA.
10:30	173	Both CD4+ and CD8+ T cells effectively suppress PRRSV replication in monocyte-derived macrophages	**C. Chung**[1], S. Cha[2], A. Grimm[1], J. Rzepka[1], D. Ajithdoss[3], G. Chung[1], W. Davis[3], C.-S. Ho[4]; [1]R&D, VMRD Inc., Pullman, WA, USA, [2]Virology division, QIA, Anyang, Korea, Republic of, [3]VMP, Washington State University, Pullman, WA, USA, [4]Gift of Life Michigan, Ann Arbor, MI, USA.

Viral Pathogenesis Los Angeles/Miami Rooms - 5th Floor Section Leader: Kyoung-Jin Yoon			
10:45	174	Both CD4+ and CD8+ T-cells recognize porcine reproductive and respiratory syndrome virus epitopes and lyse infected macrophages in a biphasic mode	**C. Chung**[1], S. Cha[2], A. Grimm[1], J. Rzepka[1], D. Ajithdoss[3], G. Chung[1], W. Davis[3], C.-S. Ho[4]; [1]R&D, VMRD Inc., Pullman, WA, USA, [2]Virology Division, QIA, Anyang, Korea, Republic of, [3]VMP, Washington State University, Pullman, WA, USA, [4]Gift of Life Michigan, Ann Arbor, MI, USA.
11:00	175	Amino acid residues Ala283 and His421 in the RNA-dependent RNA polymerase of porcine reproductive and respiratory syndrome virus play important roles in viral Ribavirin sensitivity and quasispecies diversity	**D. Tian**, X.-J. Meng; Virginia Tech, Blacksburg, VA, USA.
11:15	176	Management Practices Implemented following an outbreak of Porcine Reproductive and Respiratory Syndrome in commercial swine breeding herds in North America	J. Lowe, K. Duda; Veterinary Clinical Medicine, University of Illinois, Urbana, IL, USA.
11:30		**Lunch**	
1:30	177	Attenuation of US original porcine epidemic diarrhea virus strain PC22A via continuous cell culture passages	**C.-M. Lin**[1], S. Sommer-Wagner[1], D. Marthaler[2], Y. Hou[1], X. Gao[1], X. Liu[1], Z. Liu[1], M. S Eltholoth[1], L. Saif[1], Q. Wang[1]; [1]Department of Veterinary Preventive Medicine, Food Animal Health Research Program, Wooster, OH, USA, [2]University of Minnesota Veterinary Diagnostic Laboratory, St. Paul, MN, USA.
1:45	178	Comparison of porcine epidemic diarrhea virus (PEDV), transmissible gastroenteritis virus (TGEV) and porcine deltacoronavirus (PDCoV) for pathogenicity in weaned pigs	**K. Gibson**[1], S.M. Curry[2], E.R. Burrough[1], K.J. Schwartz[1], B. Guo[1], W.P. Schweer[2], M. Bhandari[1], H. Hoang[1], S. Azeem[1], N.K. Gabler[2], K.-J. Yoon[1]; [1]Veterinary Diagnostic and Production Animal Medicine, Iowa State University, Ames, IA, USA, [2]Animal Science, Iowa State University, Ames, IA, USA.
2:00	179	Prevalence of multi-drug resistance in E. coli and enterococci organisms isolated from abattoir workers and broilers	J.W. OGUTTU, A. Odoi; AGRICULTURE, ANIMAL HEALTH AND HUMAN ECOLOGY, UNIVERSITY OF SOUTH AFRICA, PRETORIA, South Africa, Department of Biomedical and Diagnostic Sciences College of Veterinary Medicine, University of Tennessee,, Knoxville, TN, USA.
2:15	180	A computationally designed indirect ELISA for the detection of porcine epidemic diarrhea virus (PEDV) - specific antibodies	**Y. Song**[1], P. Singh[1], E. Nelson[2], S. Ramamoorthy[1]; [1]Vet & Micro Sciences, North Dakota State University, Fargo, ND, USA, [2]Veterinary and Biomedical Sciences, South Dakota State University, Brookings, SD, USA.
2:30	181	Does systemic antibody play a role in the protection of piglets against PEDV?	K. Poonsuk, L.G. Giménez-Lirola, J. Zhang, P. Arruda, Q. Chen, L. Correa da Silva Carrion, R. Magtoto, P. Piñyero, L. Sarmento, C. Wang, K.-J. Yoon, J. Zimmerman, R. Main; Veterinary Diagnostic and Production Animal Medicine, Iowa State University, Ames, IA, USA.
2:45		**Break and Table Top Exhibits - Foyer**	
TIME	**NO**	**TITLE**	**AUTHORS**

Viral Pathogenesis Los Angeles/Miami Rooms - 5th Floor Section Leader: Kyoung-Jin Yoon			
3:00	182	Transduction of hematopoietic stem cells to stimulate RNA interference for treatment of feline infectious peritonitis	**E.A.M. Anis**[1], M. Dhar[2], R.P. Wilkes[3]; [1]Biomedical and Diagnostic Sciences, University of Tennessee, Knoxville, TN, USA, [2]Large animal clinical sciences, University of Tennessee, Knoxville, TN, USA, [3]Veterinary Diagnostic and Investigational laboratory, University of Georgia, Tifton, GA, USA.
3:15	183	Development of a snatch farrowed-colostrum deprived piglet challenge model for porcine Rotavirus C	B.N. Murphy, R. McCann, J. Lowe; University of Illinois College of Veterinary Medicine, Champaign, IL, USA.
3:30	184	Evaluation of clinical and immune responses following infection of horses with EHV-1 wild type and different EHV-1 mutants	**C.L. Holz**[1], M.E. Wilson[1], L.M. Zarski[1], R.K. Nelli[1], A. Pease[1], W. Azab[2], N. Osterrieder[2], L.S. Goehring[3], G.S. Hussey[1]; [1]Pathobiology and Diagnostic Investigation, Michigan State University, East Lansing, MI, USA, [2]Institut für Virologie, Zentrum für Infektionsmedizin â€“ Robert von Ostertag-Haus, Freie UniversitÃ¤t Berlin, Berlin, Germany, [3]Ludwig-Maximilians University, Munich, Germany.
3:45	185	Development of a real-time PCR assay for the detection and quantification of equine herpesvirus 5	**L.M. Zarski**, E.A. High, R.K. Nelli, S.R. Bolin, G. Soboll Hussey; Pathobiology and Diagnostic Investigation, Michigan State University, East Lansing, MI, USA.
4:00	186	Sites of equine arteritis virus persistence in the stallion's reproductive tract and characterization of the local inflammatory response to the virus	**M. Carossino**[1], A.T. Loynachan[2], J.R. Campos[1], B. Nam[1], I.F. Canisso[1], Y. Go[1], P.J. Timoney[1], K.M. Shuck[1], P. Henney[1], M.H. Troedsson[1], R.F. Cook[1], T. Swerczek[1], E.L. Squires[1], E. Bailey[1], U.B.R. Balasuriya[1]; [1]Veterinary Science, University of Kentucky, Lexington, KY, USA, [2]Veterinary Science, University of Kentucky Veterinary Diagnostic Laboratory, Lexington, KY, USA.
4:15	187	Equine arteritis virus uses equine CXCL16 (EqCXCL16) as a cell entry receptor	**S. Sarkar**[1], L. Chelvarajan[1], Y.Y. Go[1], F. Cook[1], S. Artuishin[1], S. Mondal[1], K. Anderson[1], J. Eberth[1], P.J. Timoney[1], T.S.
4:30	**5:00**	**Break and Table Top Exhibits - Foyer**	
5:00	**6:30**	**Poster Session II Grand Ballroom Salon III - 7th Floor**	
TUES. 8:15	188	Application of a broad-spectrum microbial detection array for the analysis of pig pathogens	**C. Jaing**[1], J. Thissen[1], S. Gardner[1], K. McLoughlin[1], P. Hullinger[1], N. Monday[2], M. Niederwerder[2], B. Rowland[2]; [1]Lawrence Livermore National Laboratory, Livermore, CA, USA, [2]Kansas State University, Manhattan, KS, USA.
8:30	189	Detection of antibody responses to the porcine circovirus strain 2 (PCV2) replicase protein.	O.H. Kolyvushko, M. Ssemadaali, X.J. Meng, S. Ramamoorthy; Veterinary and Microbiological Sciences, North Dakota State University, Fargo, ND, USA, Virginia-Maryland College of Veterinary Medicine, Blacksburg, VA, USA.

Viral Pathogenesis Los Angeles/Miami Rooms - 5th Floor Section Leader: Kyoung-Jin Yoon			
TIME	**NO**	**TITLE**	**AUTHORS**
8:45	190	Discovery of a novel putative atypical porcine pestivirus in pigs in the United States	**B. Hause**[1], E. Collin[1], L. Peddireddi[1], F. Yuan[2], Z. Chen[2], R. Hesse[1], P. Gauger[3], T. Clement[4], Y. Fang[2], G. Anderson[1]; [1]Veterinary Diangostic Laboratory and Department of Diagnostic Medicine and Pathobiology, Kansas State University, Manhattan, KS, USA, [2]Department of Diagnostic Medicine and Pathobiology, Kansas State University, Manhattan, KS, USA, [3]Department of Veterinary Diagnostic and Population Animal Medicine, Iowa State University, Ames, IA, USA, [4]Animal Disease Research and Diagnostic Laboratory, South Dakota State University, Brookings, SD, USA.
9:00	191	Identification of novel Senecavirus A from pigs with vesicular disease in the US	**B. Guo**, Y. Zheng, D. Madson, P. Gauger, P. Pineyro, C. Rademacher, D. Linhares, K. Schwartz, R. Main, K.-J. Yoon; Veterinary Diagnostic and Production Animal Medicine, Iowa State Unversity, Ames, IA, USA.
9:15	192	Protective properties of live and inactivated vaccine based on rescued recombinant influenza A virus against highly pathogenic H5N1 strain in chickens.	**A.D. Zaberezhny**[1], T.V. Grebennikova[2], G.K. Vorkunova[2], A.G. Yuzhakov[2], L.V. Kostina[2], S.N. Norkina[2], T.I. Aliper[2], E.A. Nepoklonov[3]; [1]Y.R.Kovalenko All-Russian Institute of Experimental Veterinary Medicine, Moscow, Russian Federation, [2]N.F.Gamaleya Federal Research Center for Epidemiology and Microbiology, Moscow, Russian Federation, [3]Federal Service for Veterinary and Phytosanitary Surveillance, Moscow, Russian Federation.
10:00	193	Current understanding on intercontinental HPAI: To vaccinate or not?	**C.W. Lee**; Veterinary Preventive Medicine and Food Animal Health Research Program, The Ohio State University, Wooster, OH, USA.
10:45	194	Mouse model for the Rift Valley Fever virus MP12 strain infection	Y. Lang[1], Y. Li[1], J. Lee[1], J. Henningson[1], J. Ma[1], Y. Li[1], N. Cao[1], H. Liu[1], J. Richt[1], W. Wilson[2], M. Ruder[2], S. McVey[2], **W. Ma**[1]; [1]Kansas State University, Manhattan, KS, USA, [2]Arthropod Borne Animal Diseases Research Unit, United States Department of Agriculture, Agricultural Research Service, Manhattan, KS, USA.
11:00	195	Identification and serotyping of Foot and Mouth Disease virus prevalent in Savar upazila of Bangladesh using one-step RT-PCR and multiplex RT-PCR	L. Akhter[1], **M.S. Rahman**[1], M.G. Uddin[2], M.T. Islam[1]; [1]Medicine, Bangladesh Agricultural University, Mymensingh, Bangladesh, [2]Bangladesh Livestock Research Institute, Savar, Dhaka, Bangladesh.

POSTER ABSTRACTS

Bacterial Pathogenesis

001p

001p

The role of Fur in Iron Regulation during Mycobacterium avium subsp. paratuberculosis infection - Johne's disease

F.M. Shoyama, E. Lamont, S. Sreevatsan;

Veterinary Population Medicine, University of Minnesota, Saint Paul, MN, USA.

Johne's disease (JD) is a chronic enteric infection in ruminants caused by intracellular pathogen Mycobacterium avium subsp. paratuberculosis (MAP). To reduce the prevalence of JD we need to better understand the metabolic and virulence determinants of MAP. One of the unique characteristics of MAP is that it requires supplementation with the siderophore mycobactin for optimal growth in laboratory media. Our group identified an in-vivo up-regulated Ferric uptake regulator (Fur)-like element found on the MAP-specific phage-like region as a likely candidate (MAP3773c). The objective of this study was to identify the putative function of MAP3773c in iron homeostasis. Our in silico analysis on MAP3773c as a putative Fur protein identified 23 pathways directly regulated by Fur protein, pathways that are critical for metabolism and virulence factors. Further analysis using multiple sequence alignments of well studied Fur proteins (E.coli and S. typhimurium) and MAP3773c showed 41% overall amino acid similarity with highly conserved and a nearly identical "Fur box" binding site. Based on sequence similarity and conserved "Fur" domains, MAP3773c was confirmed as a Fur-like protein that acts as a ferric uptake regulator. Using a transposon mutant of the gene (3776c) upstream of the Fur-like element (3773c) in MAP strain K10 we demonstrated concomitant knockdown of Fur transcription. We characterized the MAP3773c knockdown phenotype and validated results by restoration of Fur function utilizing a MAP3773c complementation strain. Cell invasion studies using MAC-T cells, a bovine mammary epithelial cell line, provided invasion efficiency and survival data for each MAP strain (Wild type, mutant and complement). Data showed a positive correlation between intact Fur (wild type and complement) for cell invasion. Recombinant MAP3773c was generated for protein-DNA studies through EMSA and ChIP-seq assays. These results indicate that Fur play a role in iron physiology of Mycobacterium avium subsp. paratuberculosis.

This project was supported by, MnDrive: Global Food Ventures.

Bacterial Pathogenesis

002p

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Assessment of effect of bacteriophage in post-weaning pigs challenged with enterotoxigenic Escherichia coli K88 and K99

J. Han;

College of Veterinary Medicine, Kangwon National University, Chuncheon, Korea, Republic of.

Purpose:

Introduction

The post-weaning diarrhea or colibacillosis is a most costly disease causing substantial mortality as well as growth retardation in swine production. Bacteriophages or phages have recently received reemerging attention as alternatives to antibiotics because of several merits as feed additives including their high stability within the feed and digestive tract as well as their high specificity of transfection. The present study was therefore initiated to evaluate the efficacy of dietary phages on treatment of colibacillosis induced by an oral challenge of ETEC K88 and K99 in post-weaning pigs.

Methods:

Materials and methods

Eighteen 35-d-old post-weaning pigs were allotted to three groups, after which two groups were orally challenged with 3.0 × 108 cfu of each of ETEC K88 and K99. The unchallenged group and one challenged group were fed a typical nursery diet (Control and Chal/Basal, respectively) while the remaining challenged group received the same diet supplemented with 1.0 × 109 cfu of each of ETEC K88- and K99-specific phages per kg (Chal/Phage). All animals were killed after a 7-d feeding trial and subjected to necropsy.

Results:

Results

The results of body temperature of challenged pigs are shown in Figure 1 and fecal consistency score of challenged pigs are shown in Figure 2. The ETEC K88 and K99 were detected in all feces samples obtained on d 1, 3, and 7 only in the Chal/Basal and Chal/Phage groups. The log cfu values of ETEC K88 per g feces on d 1 and 3 and per g tissue in the ileum and cecum at necropsy were less in the Chal/Phage group vs. the Chal/Basal group whereas in ETEC K99, neither the fecal excretion nor intestinal adhesion was influenced by the phage therapy.

Conclusions:

Discussion

The effect of the phage therapy appears to be significant in the ETEC K88 infection, but not in the ETEC K99 infection, in terms of suppression of intestinal adhesion and fecal excretion of the pathogens. Future studies are therefore needed to be focused on the effects of the ETEC K88-specific phage on the pathophysiological measures to further evaluate the phage as a therapeutic or prophylactic agent against porcine colibacillosis.

Bacterial Pathogenesis

003p

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Differential gene expression of mastitis-causing Escherichia coli due to swarming, swimming, and planktonic growth conditions

J.D. Lippolis[1], B.W. Brunelle[2], T.A. Reinhardt[1], R.E. Sacco[1], T.C. Thacker[3];

[1]Ruminant Disease and Immunology, National Animal Disease Center / ARS / USDA, Ames, IA, USA, [2]Food Safety and Enteric Pathogens, National Animal Disease Center / ARS / USDA, Ames, IA, USA, [3]Bacterial Diseases of Livestock, National Animal Disease Center / ARS / USDA, Ames, IA, USA.

Escherichia coli is a leading cause of intramammary infections (IMI) in dairy cattle. Typically this infection is transient in nature. However, in a

minority of cases, E. coli has been shown to cause a persistent (IMI) infection. Although the mechanisms that allow for a persistent E. coli infection are not fully understood, bacterial motility has been shown to be crucial for virulence. We have previously shown that proficient bacterial motility (swimming and swarming) is correlated with the persistent infection phenotype. In this work we look at the differential gene expression from the E. coli strains grown on swimming plates, swarming plates, and grown in culture media. Our hypothesis is that those differentially expressed genes associated with swimming or swarming are also associated with the persistent infection phenotype. Using RNA sequencing we show that genes that control or are part of the structural components of flagella, fimbria, and the iron acquisition pathways are all differentially regulated. For example, 36 genes that are associated with iron transport are up regulated in swimming bacteria compared to swarming. Understanding the mechanisms that regulate bacterial motility and its relationship to persistent IMI infections may help in the discovery of novel diagnostics and therapeutics.

Bacterial Pathogenesis

004p

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Assessment of serological effect of SUISENG® against preweaning diarrhea under field condition in South Korea

J. Han;

College of Veterinary Medicine, Kangwon National University, Chuncheon, Korea, Republic of.

Purpose:

Introduction

Escherichia coli(E. coli) and Clostridium perfringens type C(CpC) are the most important causes of diarrhea in piglets. In order to prevent economic losses from E. coli and CpC, many swine producers use inactivated vaccine in sows.

SUISENG®(Hipra, Spain) contains purified adhesion factors (F4ab, F4ac, F5 and F6), the heat labile toxin (LT) of E. coli and toxoid of CpC. The aim of this study is to assess the serological efficacy of SUISENG® on field conditions in Korea.

Methods:

Materials and methods

The study was carried out in Korean farm with 600 sows showed 15% diarrhea occurrence and around 10% mortality during lactation. The experimental groups consisted of 6 vaccinated sows and 4 control sows (30 piglets of vaccination group and 20 piglets of control group). Sera from sows, vaccinated with SUISENG® and with a placebo, and their preweaners were used in this work. Tested by using in-house ELISA provided from Hipra HQ. Mann-Whitney test and T-test($p<0.05$) of SPSS statistics 20 (IBM Corp., USA) were used for statistical significance.

Results:

Results

The mean of antibody titer against each antigens (IRPC) of the ELISA are represented in the tables 1 and 2. Tables showed clear distinction between vaccination group and control group. Sows and their piglets of vaccination group were showed higher antibody titer against all fimbrial antigens and toxin.

Statistical differences between vaccinated and control group were observed.

Conclusions:

Discussion

There was statistical significance in F4ab, F4ac, F6 and CpC antibody titers of farrowing sows. And there was significant difference in antibody titers against all antigens and toxins of 1week-old-piglets. The results clearly demonstrate that SUISENG® induces the production of specific antibodies the LT toxin and fimbrial antigens of E. coli and toxoid of CpC.

Bacterial Pathogenesis

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Effects of supplementation of lipid-encapsulated zinc oxide in weaned piglets with colibacillosis challenged with enterotoxigenic E. coli K88

J. Han;

College of Veterinary Medicine, Kangwon National University, Chuncheon, Korea, Republic of.

Purpose:

Introduction

Enterotoxigenic Escherichia coli (ETEC), which frequently causes post-weaning diarrhea, proliferates in the small intestine and is shed into feces after weaning of the piglets. Dietary supplementation of zinc oxide (ZnO) has been widely used to prevent porcine colibacillosis. Shield Zn® is a proprietary ZnO product which is encapsulated with lipid to allow the active component to reach the intestine without being ionized in the stomach. The present study was initiated to investigate the effects of dietary supplementation of 100 ppm of the coated ZnO relative to those of 2500 as well as 100 ppm of native ZnO in weaned piglets with colibacillosis induced by the ETEC K88 challenge.

Methods:

Materials and methods

Thirty-two 35-day-old weaned piglets were orally challenged with 3 × 1010 colony forming units of ETEC K88 while eight piglets received no challenge (control). Each eight challenged piglets received a diet containing 100 ppm ZnO (low ZnO), 2500 ppm ZnO (high ZnO) or 100 ppm of lipid (10%)-coated ZnO (coated ZnO) for 7 days; control pigs received the low

ZnO diet. Daily gain, goblet cell density in the villi of the duodenum, jejunum and ileum, villus height in the jejunum and ileum, fecal consistency score, serum interleukin-8 concentration, subjective score of fecal E. coli shedding, and digesta pH in the stomach, jejunum and ileum were measured.

Results:

Results

Daily gain, goblet cell density in the villi of the duodenum, jejunum and ileum, and villus height in the jejunum and ileum, which decreased due to the challenge, were greater in the coated ZnO and high ZnO groups versus low ZnO group. Serum interleukin-8 concentration, subjective score of fecal E. coli shedding, and digesta pH in the stomach, jejunum and ileum, which increased due to the challenge, were low in the coated

ZnO and high ZnO groups versus low ZnO.
Conclusions:
Discussion
Dietary supplementation of 100 ppm of lipid-coated ZnO can effectively alleviate colibacillosis in weaned piglets. Moreover, the effect of 100 ppm of the coated ZnO on colibacillosis was equal to that of 2500 ppm of native ZnO in almost all the measures associated with colibacillosis.

Bacterial Pathogenesis
006p
006p
Evaluation of SUISENG® efficacy on Korean field trial
J. Han;
College of Veterinary Medicine, Kangwon National University, Chuncheon, Korea, Republic of.
Purpose:
Introduction
Recently, Escherichia coli(E. coli) and Clostridium perfringens type C(CpC)-associated diarrhea in preweaner causes massive economic losses to the swine industry worldwide. In order to prevent economic losses from E.coli and CpC, many swine producers use inactivated vaccine. Commonly, diarrheas induced by these pathogens are prevented through sow vaccination that are booster vaccinated 2 times before farrowing. The aim of this study is to assess the serological efficacy of SUISENG®(Hipra, Spain) in commercial farm located in Korea.
Methods:
Materials and methods
The study was carried out in a farm with 300 sows in Korea showed 15% occurrence of diarrhea and around 10% mortality during lactation. The experimental groups consisted of 6 vaccinated sows and 4 control sows (30 piglets of vaccination group and 20 piglets of control group). Sera from sows, vaccinated with SUISENG® and with a placebo, and their preweaners were used in this work. Tested by using in-house ELISA provided from Hipra HQ.
Mann-Whitney test and T-test($p<0.05$) of SPSS statistics 20 (IBM Corp., USA) were used for statistical significance.
Results:
Results
The mean of antibody titer against each antigens (IRPC) of the ELISA are represented in the Tables 1 and 2. Tables showed clear distinction between vaccination group and control group. Sows and their piglets of vaccination group were showed higher antibody titer against all fimbrial antigens and toxin.
Statistical differences between vaccinated and control group were observed.
Conclusions:
Discussion
There was statistical significance in F4ac, F5.antibody titers of farrowing sows. And there was significant difference in antibody titers against CpC, F4ab, F5, F6 and LT of 1week-old-piglets. The results clearly demonstrate that SUISENG® induces the production of specific antibodies the LT toxin and fimbrial antigens of E. coli and toxoid of CpC.

Bacterial Pathogenesis
007p
007p
Vaccination to prevent brucellosis in elk (*cervus canadensis*).
S. Olsen;
National Animal Disease Center, Ames, IA, USA.
Brucella abortus is a bacterial pathogen of economic importance that causes abortions and reproductive losses in large ruminants, and zoonotic infection in humans. Regulatory programs were initiated over 80 years ago and, at the present time, the disease is essentially eradicated in cattle.in the U.S. The perisistence of Brucella infection in bison and elk in the area in and surrounding Yellowstone National Park pose a risk for reintroduction to domestic livestock. In a series of studies, we evaluated the immunologic responses of elk to vaccination with the B. abortus strain RB51 (RB51) vaccine alone, or in combination with adjuvants. Elk inoculated with RB51 had greater ($P<0.05$) humoral responses to the vaccine strain as compared to non-vaccinated elk. In a similar manner, co-administration of one adjuvant with RB51 increased ($P<0.05$) lymphocyte proliferative responses after vaccination. Elk were experimentally challenged in midgestation with strain 2308 by conjunctival administration. Incidence of abortion, and incidence of infection with the challenge strain at parturition did not differ ($P>0.05$) between vaccination and control treatments. Our data suggest that elk are more resistant to experimental infection than cattle or bison, and that the vaccination treatments evaluated in the current study did not increase protection against brucellosis.

Bacterial Pathogenesis
008p
008p
Targeting methicillin resistant Staphylococcus pseudintermedius (MRSP) with novel antimicrobial peptides
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Staphylococcus pseudintermedius is a major cause of skin and soft tissue infections in companion animals and has zoonotic potential. Additionally, methicillin-resistant S. pseudintermedius (MRSP) has emerged with resistance to virtually all classes of antimicrobials. Thus, novel treatment options with new modes of action are required. Here, we investigated the antimicrobial activity of six synthetic short peptides against clinical isolates of methicillin-susceptible and MRSP isolated from infected dogs. All six peptides demonstrated potent anti-staphylococcal activity regardless of existing resistance phenotype. The most effective peptides were RRIKA (with modified C terminus to

increase amphipathicity and hydrophobicity) and WR-12 (α-helical peptide consisting exclusively of arginine and tryptophan) with minimum inhibitory concentration50 (MIC50) of 1 μM and MIC90 of 2 μM. RR (short anti-inflammatory peptide) and IK8 "D isoform" demonstrated good antimicrobial activity with MIC50 of 4 μM and MIC90 of 8 μM. Penetratin and (KFF)3K (two cell penetrating peptides) were the least effective with MIC50 of 8μM and MIC90 of 16 μM. Studies with propidium iodide and transmission electron microscopy revealed that peptides damaged the bacterial membrane leading to leakage of cytoplasmic contents and consequently, cell death. A potent synergistic increase in the antibacterial effect of the cell penetrating peptide (KFF)3K was noticed when combined with other peptides and with antibiotics. In addition, all peptides displayed synergistic interactions when combined together. Furthermore, peptides demonstrated good therapeutic indices with minimal toxicity toward mammalian cells. Resistance to peptides did not evolve after 10 passages of S. pseudintermedius at sub-inhibitory concentration. However, the MICs of amikacin and ciprofloxacin increased 32 and 8 fold, respectively; under similar conditions. Taken together, these results support designing of peptide-based therapeutics for combating MRSP infections, particularly for topical application.

Bacterial Pathogenesis

009p

009p

Inter- and intra-serotype differences in the uptake, survival and modulation of nitric oxide production in avian macrophages (HD-11) by the most prevalent poultry-associated Salmonella seroTypes

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Purpose:

Contaminated poultry meat and eggs is an important source of human salmonellosis. Certain serotypes are more frequently and consistently isolated from marketed poultry meat in the US. The objective of this study was to determine the inter- and intra-serotype differences in the uptake/invasion, survival and modulation of nitric oxide in avian macrophages (HD-11) by genetically diverse isolates of Most Prevalent Poultry-associated Salmonella seroTypes (MPPSTs).

Methods:

Ninety isolates of MPPSTs isolated from the US poultry sources during 1990 to 2013 were tested. These MPPSTs included Kentucky (n=10), Mbandaka (n=10), 1,4[5],12:i, (n=9), Enteritidis (n=8), Montevideo (n=8), Senftenberg (n=8), Heidelberg (n=7), Hadar (n=7), Infantis (n=7), Thompson (n=7), Typhimurium (n=6) and Schwarzengrund (n=3).

Results:

PFGE showed that each serotype formed individual clusters, except Typhimurium and 1,4[5],12:i which clustered together. Most isolates within each cluster were genetic distinguishable. Isolates of Enteritidis and Hadar were genetically clonal (91% similarity), whereas Senftenberg were diverse (59% similarity). The mean invasion of HD-11 cells was highest for Hadar (3.6%) and lowest for Senftenberg (1.5%). The mean intracellular survival was highest for Schwarzengrund (8.1%) and lowest for Heidelberg (0.6%). Senftenberg invaded HD-11 cells less efficiently (1.5%), but showed higher intracellular survival (3.1%). In contrast, Schwarzengrund showed higher invasion (3.35%) and higher intracellular survival (8.1%) whereas Kentucky showed low invasion (1.7%) and low intracellular survival (1.15%) in avian macrophages. Few isolates of Schwarzengrund (2), Enteritidis (2), Senftenberg (2), Montevideo (2), Thompson (1), Infantis (1) and Mbandaka (1) induced significantly low levels of NO.

Conclusions:

These data demonstrate that there are inter- and intra-serotype differences in the invasion and survival of MPPSTs in avian macrophages and induction of nitric oxide. Further studies are needed to investigate if this differential intracellular survival correlates with their pathogenicity in chickens and human.

Bacterial Pathogenesis

010p

010p

Preliminary sequence comparisons of six Leptospira strains

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Leptospirosis is an emerging infectious and a widespread zoonotic disease caused by pathogenic members of the genus Leptospira. This disease is enigmatic as many animal species asymptomatically harbor the organism in the renal tubules and severe disease may occur in humans and animals during accidental infections. Next generation sequencing (NGS) technology is a valuable tool for studying genomic structures, prediction of molecular signatures and new hypothesis driven exploratory research, thus facilitating expansion of knowledge about bacterial pathogens. In this study, five Leptospira borgpetersenii isolates and one Leptospira interrogans isolate belonging to the serovar Hardjo were sequenced using next generation sequencing using the Illumina platform. The sequences were de novo assembled using SPAdes genome assembler and deposited into the web based Rapid Annotations using Subsystems Technology (RAST) server. The comparison between subsystem category distributions within genomes of isolates showed a notable difference in RNA metabolism; cofactors, vitamins, prosthetic groups, pigments and membrane transport subsystems. Subtle differences between subsystems in RNA metabolism, amino acids, nitrogen metabolism, fatty acids, lipids and isoprenoids were also observed. The classes of genes that differ between Leptospira borgpetersenii and Leptospira interrogans, included genes involved in protein biosynthesis, RNA processing/metabolism, DNA metabolism and stress response. Alternative annotation methods will be implemented in future analysis to increase a chance to discover putative virulence factors, potential antibiotic resistance genes and other genetic elements or novel pathogenicity descriptors that might be missed during RAST based annotations/analysis.

Bacterial Pathogenesis

011p

011p

Risk analysis of Q-fever entry in the territory of Ukraine

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Coxiella burnetii is an obligate intracellular bacterium that causes Q fever in animals and humans. The epizootic situation of Q-fever in border countries (Poland, Slovakia, Hungary, and Romania) leads to a constant current risk of Q-fever entering Ukraine. There is the possibility of infection of humans and animals in the absence of direct connection with the source of infection and permanent migration of the working population to neighboring countries.

The aim of this study is to conduct risk analysis on the possibility of Q-fever spread to domestic animals and the Ukrainian people from the European border countries.We performed analysis of data from OIE and EFSA (World Organization for Animal Health and European Food Safety Authority) reports about the spread of Q-fever during 2008-2013. Research on animal infection with Q-fever was conducted in the 10 oblasts of Ukraine. Ukraine borders Poland, Slovakia, Moldova, Romania and Hungary. These countries are reporting infections of Q-fever in humans and animals. A total of 1180 sera from domestic animals were examined from 10 oblasts of Ukraine during 2011 - 2015, and 784 were from Odessa oblast during 2011 - 2014. Between 2011-2014, a total of 93 samples were assayed and 16 were seropositive (17,2%). All samples, including seropositive samples, were tested by PCR and tested negative. Geographic distribution analysis showed that in the Odessa oblast there is one natural foci of Q-fever registered in three southern rayons (Artsizsky, Kiliyskiy, and Tatarbunarsky rayons). Monitoring study results have shown that samples from farm animals taken from Sumy, Kharkov, Donetsk, Kyiv, Lugansk, Chernovtsy, Kherson, Dnepropetrovsk, Ternopol, Ivano-Frankovsk, and Odessa oblasts did not contain antibodies against C. burnetii and are negative for C. burnetii DNA.

1. Of particular risk to Ukraine are the border countries which have epizootic areas of Q-fever, and which border the western regions of Ukraine, where most cattle are concentrated, both in households and on farms.
2. To identify and prevent the spread of disease in Ukraine, it is vital to conduct monitoring of domestic animals.

Bacterial Pathogenesis

012p

012p

Comparative genomic analysis of two netF-positive Clostridium perfringens isolates associated with foal necrotizing enteritis and canine haemorrhagic gastroenteritis.

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A role for type A *C. perfringens* in diarrhea and enteric disease of dogs and foals has long been suspected but incompletely characterized. However, the recent discovery of a novel beta-pore-forming toxin, NetF, which is strongly associated with canine and foal necrotizing enteritis should improve our understanding of the role of type A strains in disease in these animals. This study presents the complete genome sequence of two *netF*-positive *C. perfringens* strains, JP55 and JP838, recovered from a case of foal necrotizing enteritis and canine haemorrhagic gastroenteritis, respectively. Genome sequencing was done using Single Molecule, Real-Time (SMRT) technology-PacBio and Illumina Hiseq2000.The JP55 and JP838 genomes include a single 3.34 Mb and 3.53 Mb circular chromosome, respectively, and both genomes additionally consist of five circular plasmids. Comparison of these two *C. perfringens* chromosomes with three fully sequenced reference chromosome sequences identified 48 (~ 247 kb) and 81(~ 430 kb) unique genomic regions for JP55 and JP838, respectively. Some of these divergent genomic regions are phage- and plasmid-related segments. Sixteen of these unique regions (~69 kb) were however shared between the two isolates. Five of these common regions formed a mosaic of plasmid-integrated segments, suggesting that these elements were acquired early in a clonal lineage of *netF*-positive *C. perfringens* strains. In addition, plasmid annotation revealed that both *netF*-positive *C. perfringens* strains, JP55 and JP838, harbour three plasmids in common, including a NetF/NetE toxins-encoding plasmid, a CPE/CPB2 toxins-encoding plasmid and a putative bacteriocin-encoding plasmid. We also found that JP55 and JP838 strains, which are associated with foal necrotizing enteritis and canine hemorrhagic gastroenteritis, share unique virulence genes on conserved pathogenicity loci on the large *tcp*-conjugative plasmids. These results provide significant insight into the pathogenetic basis of canine and foal necrotizing enteritis and are the first to demonstrate that *netF* resides on a large and unique plasmid-encoded locus.

Biosafety and Biosecurity

013p

013p

Status of laboratory biosafety and biosecurity in veterinary research facilities in Nigeria

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[1]Department of Veterinary Public Health and Preventive Medicine, University of Ilorin, Ilorin, Nigeria, [2]Department of Veterinary Pathology, University of Ibadan, Ibadan, Nigeria, [3]Department of Virology, University of Ibadan, Ibadan, Nigeria, [4]Viral Research Division, National Veterinary Research Institute, Vom, Nigeria, [5]Department of Food Science, University of British Columbia, Vancouver, BC, Canada, [6]National Biosafety and Biocontainment Training Program (NBBTP), National Institutes of Health, DOHS, Bethesda, MD, USA.

Purpose: This study was conducted to determine the status of laboratory biosafety and biosecurity in Nigerian veterinary research facilities.

Methods: A questionnaire was designed and pretested to obtain information from researchers working in veterinary facilities across Nigeria from July 2014 to July 2015. Information on demographic factors, knowledge of laboratory biosafety, availability and proper use of biosafety devices and personal protective equipment (PPE), priority pathogens researched, attitude on and use of standard laboratory practices, and biosafety awareness were obtained using a numeric scoring system. Data were analyzed using descriptive statistics and factors predicting the status of laboratory biosafety were determined using logistic regression.

Results: A total of 74 participants completed the questionnaires. Respondents had a mean age of 35.7±6.9 years. A majority (68.9%) were less experienced having spent <10 years at work. General knowledge scores ranged from 3 to 28, of 28 possible points and majority (94.6%) had low

scores (scores < mean+1SD). Few (17.6%) reported availability and good usage of biosafety devices and PPE. Many (63.5%) have no access to biosafety level 1-3 facilities and none reported the availability of a BSL-4 facility. Individuals reporting a lack of access to biosafety cabinets in their research facilities was high (67.6%). Knowledge scores pertaining to management practices ranged from 0 to 14, of 14 possible points, and was positively skewed (mean =3.84, median =3.00) and 47.3% had good scores (scores < mean+1SD). Only 16.2% have biosafety officers in their research facilities. Few (n=8) reported working on highly infectious viral pathogens in risk group 4 (Pesti des petits ruminants, Lassa fever and African swine fever viruses). Rabies virus was the most researched pathogen (31.1%). A majority (71.6%) were unaware of national laws guiding biosafety in Nigeria and globally. Certain occupations and facility types were significantly associated ($p< 0.05$) with good laboratory biosafety scores in Nigerian veterinary facilities.
Conclusions: Occupation and facility type could serve as indicators of status of laboratory biosafety.

Biosafety and Biosecurity
014p
014p
Development of real-time PCR methods for rapid detection and quantification of two marine harmful algal bloom microalgae: Karenia mikimotoi and Prorocentrum donghaiense
J. Yuan[1], T. Mi[2], Y. Zhen[2], Z. Yu[3], S. Ensley[1], K.-J. Yoon[1];
[1]Department of VDPAM, College of Vet Med, Iowa State University, Ames, IA, USA, [2]Department of Environmental Sciences, College of Environmental Science and Engineering, Ocean University of China, Qingdao, China, [3]Department of Marine Chemistry, College of Chemistry and Chemical Engineering, Ocean University of China, Qingdao, China.
Harmful Algal Blooms (HABs) are globally important issues for both marine and freshwater environments. These can have great adverse effects on the aqua-ecosystems accountable for economic loss of more than US$300 million and cause considerable harms to animal and human health. Karenia mikimotoi and Prorocentrum donghaiense are both marine HABs microalgae which have resulted in serious blooms also known as “red tides”. These microalgae have led to severe destruction in marine ecosystems and great losses in aquaculture. Toxins released by K. mikimotoi are potential threats to humans. At present, there are no effective ways to diminish the harms of HABs once occurred. Therefore, it is crucial to detect them quickly, preferably before their occurrence. In this study, real-time PCRs (qPCRs) were developed for rapid detection and quantification of K. mikimotoi and P. donghaiense. Targeting their ITS (Internal Transcribed Spacer) regions, species-specific primers and probes were designed to increase the specificities of detection. Lab-cultured algal samples and recombinant plasmids containing target fragments were also prepared as standard samples in qPCR. The PCR assays were able to quantify as few as 102 cells for both algae. On field samples from the East China Sea, K. mikimotoi was detected by the qPCR at a low concentration (147 ± 20 cells per liter). The methods were also successfully applied in an investigation of a P. donghaiense bloom in the East China Sea in accordance with microscopic examinations. In conclusions, the newly developed PCR methods are useful for the rapid detection and quantification of the two HABs microalgae. A similar approach can be used for other HABs associated with in-land water bodies, such as blue-green algae blooms.

Biosafety and Biosecurity
015p
015p
Bluetongue, Akabane and Schmallenberg virus surveillance in Kazakhstan
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[1]National Reference Veterinary Center, Astana, Kazakhstan, [2]Naval Medical Research Center, Frederick, MD, USA.
Scientists of the Republic of Kazakhstan National Reference Veterinary Center (NRVC) and United States Naval Medical Research Center (NMRC) evaluated archived bovine sera for the presence of Bluetongue virus, Akabane virus, and Schmallenberg virus, three reemerging arboviruses that can affect livestock in Kazakhstan.
Serum samples are collected when farm animals are imported into the country, as well as during any suspected disease outbreak. Under this study, we identified a total of 777 retained serum samples collected from domestic farm animals in different regions of Kazakhstan as well as from cattle imported to Kazakhstan in 2012.
We utilized commercial ELISA test kits to identify antibodies to Bluetongue and Akabane and Schmallenberg viruses. Samples that tested positive for Schmallenberg antibodies by ELISA were tested for the presence of Schmallenberg RNA by quantitative real-time PCR (qPCR) using Life Technology (France) test kits. Results were mapped using ArcGIS.
The history of the samples studied under this project provides insight into the project’s results. Originally, sera were taken from two different lots of cattle - from Austria and from Kazakhstan. Austrian animals were sampled when they were brought into the country, which at the time resulted in the culling of a significant number of animals due to detection of Schmallenberg virus. The samples account for the majority of positive test results under this project (56). Positive serum samples of Kazakhstan animals could be a result of carry-over from infected animals imported to Kazakhstan. Regardless, the results indicate a potential presence of Schmallenberg virus in Kazakhstan that warrants further study. Although the ELISA detected antibodies, the PCR failed to detect Schmallenberg RNA because of the short viraemic period of the virus (2-6 days).
A low number of Blutongue virus-positive samples (6) was also identified; very little is known about the distribution of *Culicoides* in Kazakhstan, and there is no contributing history on animal movement to provide insight. Further investigation is needed among sheep and goats in the area.

Companion Animals Epidemiology
016p
016p
Spatial and temporal distribution of brucella isolates recovered from slaughtered cows in Egypt during the years of 2010-2015
H.H. Abdalaal, Sr.;
Veterinary Medicine, Beni Suef university, Beni Suef, Egypt.
In this study, different tissues specimens of 182 cows including spleen, retropharyngeal and supra-mammary lymph nodes and udder of

brucella seropositive cows during the years of 2010-2015 were subjected for bacteriological and molecular investigations for detection and identification of Brucella organisms. Tissue specimens were subjected for DNA extraction, conventional PCR and multiplex PCR for detection of Brucella infection. Amplification of target gene (Immunodominant antigen, gene bp26) and Bruce-ladder were carried out for molecular identification of Brucella in DNA extracts of tissue specimens. Amplification of target gene (Immunodominant antigen, gene bp26) revealed positive results for the genus Brucella in all DNA extracts. Results of the Bruce-ladder revealed Brucella melitensisin in all DNA extracts. Bacteriological examinations revealed isolation of Eighty five Brucella isolates. All cultures were smooth and colonies were elevated, transparent, and convex, with intact borders, brilliant surface and have a honey color under transmitted light. In this study, characterization at the biovar level of the 85 Brucella field isolates were carried out. The criteria used were requirement for additional atmospheric 10% CO2, production of hydrogen sulphide gas, production of urease, growth on media containing the inhibitory dyes thionin and basic fuchsine, agglutination with monospecific antisera A, M and R and employing three stable Brucella phages. The obtained results showed that all the 85 isolates were typed as Brucella melitensis biovar 3. Brucella isolates were distributed in eight Governorates; Beni Suif Governorate (32 isolates), Al Faium Governorate (20 isolates), Assiut Governorate (eight isolates), Kafr El-Shikh Governorate (seven isolates), Damietta Governorate (seven isolates), Dakahlia Governorate (six isolates), and Menofia Governorate (five isolates). The sensitivity of the PCR was generally considered to be higher than bacterial isolation. Brucella melitensis biovar 3 still remains the sole and predominant Brucella organism affecting Egyptian cattle which constitutes major threat to livestock as well as humans.

Companion Animals Epidemiology

017p

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Spatial and temporal distribution of brucella isolates recovered from slaughtered cows in egypt during the years of 2010-2015.h.i.hosein1, a.menshawy2, sherin r. rouby3 and ayman mahrous4. 1,2,3 dept. of veterinary medicine, college of veterinary medicine, beni suef university, egypt. 4 govs, giza, egypt.

H.I.H. Abdalaal, Sr., A.M. Menshawy, S.R. Rouby;
Veterinary Medicine, Beni Suef university, Beni Suef, Egypt.

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Companion Animals Epidemiology

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Companion Animals Epidemiology

019p

019p

Bacteriological and molecular characterization of Brucella melitensis in Dromedary camels in Egypt

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This study was undertaken to determine the sero-prevalence of the disease in camels, in Egypt. A serological study using 1126 blood samples collected from Dromedary camels was carried out. The modified Rose Bengal Plate (mRBPT) Test and competitive ELISA (cELISA) were used as screening and confirmatory tests, respectively. The overall sero-prevalence of Brucella antibodies was 4.00% and 3.7.00% detected by the RBPT and c-ELISA respectively. Lymph nodes, testicular tissues, udder tissues and milk from seropositive positive camels were used for isolation of Brucella organisms. Brucella melitensis biovar 3 was isolated from milk of two she camels and lymph nodes of four male camels. The bacteriological findings suggested that camels were infected from cattle or sheep and goats where Brucella melitensis biovar 3 is prevalent in Egypt. The mRBPT was suitable for screening camel sera for brucellosis, but the cELISA was mor specific. It is likely that the tendency to raise large flocks of sheep along with the camels contributed towards the spread of Brucella melitensis among camels. DNA extract of two milk samples from which Brucella melitensis was isolated was tested using Bruce ladder multiplex PCR. The test has amplified three fragments of 587 bp, 1071 bp and 1682 bp sizes confirming our bacteriological finding. Our findings support the power of PCR testing for Brucella spp. detection and could be used effectively in routine diagnosis of brucellosis.

Companion Animals Epidemiology

020p

020p

Evidence of Mycobacterium bovis in a dog population that cohabits with cattle

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Mycobacterium bovis is the etiological agent of bovine tuberculosis. Presence of this pathogen in different animal species and humans is well described. However, information in dogs is limited, therefore in this study we aimed to identify presence of *Mycobacterium bovis* in a dog population that lives in close contact with cattle naturally exposed to this pathogen. We conducted our study in a complex that houses 26,000 dairy cattle with 16% prevalence of tuberculosis. Canine population in this complex is composed of 247 dogs. Dogs had free access to all facilities in the complex, therefore they were in close contact with cattle and had access to raw milk and animal tissues like placenta and fetuses. In order to identify risk factors associated to *Mycobacterium bovis* infection we conducted an epidemiological survey. Sixty five dogs were selected at random to perform general physical examination, tuberculin skin testing (TST) and interferon gamma release assay (IGRA). Base on our results, we selected a subgroup of dogs (19) for X-ray and necropsy (1) examination. Dogs remaining free (not tied) (OR 4.9) and those with access to all facilities (OR 19.35) had more probability to get a positive result on the IGRA test. Results on the TST were ambiguous having reactions between 1 to 13 mm; 12.4 % of the population had a reaction over 5 mm; however there was no correlation with results from the IGRA test (67.69 % of dogs with positive result). X-ray results suggested that 5 dogs had pneumonia. At necropsy, macroscopic examination showed multifocal white nodules on the lung lobes. Histopathology results showed interstitial pneumonia and reactive lymph nodes, Ziehl Neelsen staining showed material with tinctorial affinity of mycobacteria. A positive PCR result (MPB70) was obtained from tissues collected at necropsy. We have evidence supporting the presence of *M. bovis* in a dog population that lives together with cattle. This work was supported by project CONACYT CB-167488.

Companion Animals Epidemiology

021p

021p

Monitoring of circulation of H1, H2, H3 “human” influenza viruses among wild birds in different ecological groups of Ukraine in 2006-2012

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Currently, influenza is one of the most dangerous and unpredictable disease in humans, animals and birds. Influenza virus subtypes H5, H7 and some others cause certain danger for human health. One should also remember “classical” human influenza viruses subtype H1, H2 and H3 that continuously circulate in natural reservoir. Under the specific conditions they might be gene donors for new epidemic viruses. Therefore, we studied the peculiarities of the circulation of influenza virus H1, H2 and H3 in wild birds of different ecological groups in Ukraine.

During monitoring of influenza virus among wild birds, 27 families were studied в 2006-2012 in different regions of Ukraine. Serological methods were used to test 946 wild birds and by virology methods - 6281 wild birds.

Due to the results of serological tests we obtained the following data: in 2006 6.06% of Dunlins, 5.88% Grey Plovers, 14.28% of Curlew Sandpipers, 40% of Broad-billed Sandpipers, in 2008 15.38% of Mute Swans and in 2011 6.97% of Mallards had antibodies to AIV H1 in blood serum. Antibodies were also detected in the egg yolk of Song Thrush and Jay in 2006, in 3.22 - 4.16% Mallard yolk in 2009. Antibodies to H2 were detected in the serum of 13.04%, 1.61%, 1.55 -3.04% of Mallards in 2006, 2009 and 2011 respectively and in 2006 - in egg yolks of Song Thrush, Great Tit, Slender-Billed Gulls and in egg yolks of Mallards (12.5% positive samples). In 2006 antibodies to H3 were detected in egg yolk of Yellow-legged Gull (23.07% positive samples), in 2007 - in Coot’s yolks (33.3%) and Grey Plover’s yolk (14.28%), in 2008 - in the yolks of Common Terns (single sample) and in 2009 роцi - in the blood serum of Mallards (12.90%).

According to the results of virology studies of 6281 wild birds, 98 viruses were isolated, 23 of them belonged to APMV and 69 - AIV. Totally, 16

viruses subtype H1, H2 and H3 were isolated(H1N1 - 7 isolates, H1N2 - 1, H3N8 - 5, H2N3 - 2, H2N? - 1).
Obtained data show quite a wide circulation of H1, H2 and H3 "human" influenza viruses among swimming birds. Special attention should be paid to the circulation of influenza virus H2 in natural reservoirs, as it has been registered in humans for many years.

Epidemiology and Animal Economics

022p

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Environmental surveillance for bacterial extended spectrumβbeta-lactamase resistance genes at a municipal wastewater treatment plant

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In response to ever increasing use of antibiotics, bacteria are evolving resistance to critical frontline antimicrobial drugs that treat invasive Gram-negative infections. The most serious threat is bacteria that are resistant to carbapenem drugs. Bacteria may gain this resistance by acquiring mobile resistance genes that confer the ability to produce enzymes that inactivate the antibiotic. Numerous genes, including the blaKPC and blaNDM-1 are known to encode bacteria the ability to produce cabapenemase. While both are present in the US, blaKPC has emerged and disseminated primarily in the US, while blaNDM-1 has primarily disseminated in SE Asia. Because of the frequency of international travel we hypothesized that both blaKPC and blaNDM-1 could be present in Ohio waste-water treatment plants. The purpose of this study was to determine if carbapenem-resistant E. coli were present in Columbus wastewater, and to fully characterize those isolates and their resistance mechanisms. We collected 334 samples of untreated sewage water at the Jackson Pike Wastewater Plant between June and August of 2011 and 2012. Using selective media, we identified 158 (47.3%) samples with suspect colonies that grew in the presence of 1 mg/L of meropenem. Of these, 51 (32.9%) were classified as meropenem resistant using Kirby-Bauer disk diffusion assay and 29 isolates were also confirmed to be E. coli using biochemical tests and PCR. These isolates were resistant to most of the 26 drugs on our MIC panels using micro-broth dilution. Carbapenemase production was verified for 76 isolates using the Modified Hodge test. However, none of the isolates were positive on the EDTA Double Disk Diffusion test, indicating absence of metallo-β-lactamase production. Our detection of these isolates suggests the presence of a reservoir of important resistance genes for pathogens. Surveillance is an important component of education, awareness, and prevention of antimicrobial resistance in the public health sector.

Epidemiology and Animal Economics

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Antimicrobial susceptibility profiles of MRSA ST5 isolates from swine production settings and clinical isolates from humans with no swine contact

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Purpose: Livestock associated methicillin-resistant *Staphylococcus aureus* (LA-MRSA) draws public health concern as these isolates represent the largest reservoir of MRSA outside hospital settings. Recent studies indicate LA-MRSA from swine are more genetically diverse than the first reported sequence type ST398. In the US, LA-MRSA is a mixed population of ST398, ST9, and ST5. Identification of ST5 is of concern since ST5 is a highly successful and widely disseminated human-associated lineage. This success results in part from acquisition of mobile genetic elements carrying antibiotic resistance genes. Resistance elements are a major concern in MRSA and resistance to higher tier antibiotics has been reported. Because of increased exposure to antibiotics in a hospital setting, we hypothesized clinical MRSA isolates would be resistant to more antibiotics than LA-MRSA isolates.

Methods: 155 MRSA ST5 isolates were obtained from swine, swine facilities, humans with short or long-term swine exposure, and clinical isolates from humans with no swine contact. Minimum inhibitory concentrations to 28 antibiotics (14 classes) were determined by microbroth dilution on GPALL1F and BOPO6F plates in accordance with Clinical Laboratory Standards Institute recommendations.

Results: LA-MRSA ST5 isolates were resistant to significantly fewer antibiotics than human clinical ST5 isolates, average 11.7 vs 14.4 antibiotics, respectively. All MRSA ST5 isolates tested were susceptible to the higher tier antibiotics linezolid and vancomycin. Significantly more LA-MRSA ST5 isolates were resistant to tetracycline class antibiotics than human clinical isolates, while significantly more clinical isolates were resistant to fluoroquinolone and macrolide antibiotics.

Conclusions: The susceptibility profiles in this study are consistent with the hypothesis that clinical MRSA isolates are resistant to more antibiotics than LA-MRSA isolates. The resistance profiles of the LA-MRSA isolates were also consistent with antibiotic usage in animal agriculture, with a high proportion resistant to tetracycline class antibiotics and fewer resistant to fluoroquinolone antibiotics.

Epidemiology and Animal Economics

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Dissemination of antimicrobial resistant enteric bacteria in a zoo environment

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Dissemination of antimicrobial resistant enteric bacteria in a zoo environment

Sydnee M Feicht, Dimitria A. Mathys, Dixie F. Mollenkopf, Thomas E. Wittum

Objective: Antimicrobial resistant bacteria in the environment pose a potential health hazard to both the public and the zoo animal population. Our aim is to determine the prevalence of extended-spectrum beta-lactam and fluoroquinolone resistant Enterobacteriaceae and Salmonella

on surfaces at public and animal areas of a large zoo in Ohio.
Methods: Separate electrostatic cloths were used on flat surfaces of human and animal contact, and then enriched in nutrient broth with 2 ug/ml cefotaxine or 16 ug/ml naladixic acid. The cefotaxine broth was inoculated onto MacConkey Agar with 8 ug/ml of cefoxitin, 4 ug/ml of cefepime, or 2 ug/ml of meropenem, to identify the blaCMY, blaCTX-M, and carbapenemase phenotypes. The naladixic acid broth was inoculated onto MacConkey Agar with 2 ug/ml of ciprofloxacin or 16ug/ml naladixic acid, to identify fluoroquinolone resistant phenotypes. A third cloth was enriched in buffered peptone water, then Rappaport-Vassiliadis broth, and then inoculated onto an XLT-4 plate for the isolation of Salmonella.
Results: Phenotypic blaCMY isolates were found on 28.6% of animal only surfaces, 25% of human only surfaces and none on animal and human surfaces. Phenotypic blaCTX-M isolates were found on 35.7% of animal only surfaces, 7.1% of human only surfaces, and 17.9% of human and animal surfaces. Naladixic acid resistant isolates were found on 71.4% of animal only surfaces, 25% of human only surfaces, and 50% of human and animal surfaces. Ciprofloxacin resistant isolates were found on 21.4% of animal only surfaces, 32.1 of human only surfaces and 41.7% of human and animal surfaces.
Discussion: Our results suggest that the zoo environments harbor bacteria resistant to clinically important antimicrobials which pose a potential risk to the public, zoo staff, and animal health. The zoo environment provides the opportunity for a diverse population of humans and animals to be exposed to coliform bacteria expressing multiple antimicrobial resistant phenotypes.

Epidemiology and Animal Economics

025p

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Longitudinal monitoring of small layer flocks with less than 3,000 hens for Salmonella Enteritidis

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Foodborne outbreaks of human salmonellosis have been traced back to the consumption of Salmonella Enteritidis (SE)-contaminated chicken eggs and egg products. Since July 2012, Food and Drug Administration (FDA) mandated shell egg producers with more than 3,000 hens to take measures to prevent SE in shell eggs during production, storage, and transportation. Flocks with less than 3,000 hens represent 1% of the total egg production in United States are not required to follow the FDA guidelines with the assumption that the small producers sell their eggs directly to the consumers or do not produce shell eggs for the table market. Objective of this study was to estimate the SE incidence in flocks with less than 3,000 hens and to characterize SE isolated from these flocks to assess their relatedness to SE isolated from human foodborne outbreaks in the United States. This study monitored SE contamination of eggs produced in 40 small layer flocks in two major egg producing states in the United States, Pennsylvania and Iowa. Samples were tested for the presence of Salmonella by culture at the age of day 1 (chick box papers) and weeks 15, 30, and 45 (hen house drag swabs, feed, water, rodents, insects), and eggs if environmental samples were positive for SE. Salmonella recovered were serotyped and any SE were further characterized by phage typing and pulsed-field gel electrophoresis. Hen house environmental samples from five out of 40 flocks were positive for SE. And, SE was present in the eggs from four of the five environmental positive flocks. Rodents were positive for SE only in one flock. Typing methods demonstrated SE found in the hen house environment, rodents, and eggs were either identical or highly similar. The most common phage type found was type 8 which is also the major phage type associated with human Salmonella outbreaks in the United States. These findings highlight the risk associated with SE contaminated eggs produced in flocks with less than 3,000 hens that are currently exempted from the FDA's Final Rule to take measures to prevent SE contamination of eggs.

Epidemiology and Animal Economics

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Investigation of biofilm producing ability in *Staphylococcus* spp. isolated from buffalo milk and milking environment using phenotypic and genotypic assays

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The ability to escape host immune defenses by biofilm formation and the presence of other virulence factors are thought to be key elements in intra-mammary infection. The aim of this study was to characterize the ability of *Staphylococcus* spp. isolates from buffalo milk and milk environment to produce biofilm and to determine the presence of several known virulence genes. After the mammary gland physical examination, 320 milk samples (80 animals X 4 quarters) were evaluated using the strip cup test and California Mastitis Test (CMT). In addition, hand samples (n= 16) from consenting milkers and from milking machines (n=32) were collected with sterile swabs. After initial classification by Gram staining and biochemical testing, coccobacillary isolates that were Gram positive, catalase and coagulase positive, were further characterize using genus specific (TStaG 422 forward and TStaG 765 reverse) PCR primers. Thirty-two *Staphylococcus* spp. isolated from buffalo milk, milking machines, and milker hands were tested for presence of the *ica*A, *ica*D, *clf*A, *clf*B, *sar*A, and *hla* genes, slime production on Congo Red Agar (CRA), and biofilm formation in microtitre plates using a crystal violet assay. All samples produced biofilm. Nine (28.1%) of the 32 isolates were positive for the presence of both *ica*A and *ica*D genes. No isolates were positive for *ica*A but not *ica*D; seven (21.8%) isolates were positive for only *ica*D. In this study, 17 strains had the *sar*A gene and also carried the *clf*A, *clf*B, or *hla* genes. In the absence of the *sar*A gene, *clf*A, *clf*B, or *hla* were not present. The *hla* gene was also detected in 17 isolates. Given that the *hla* gene is associated with staphylococci that cause food poisoning, the pathogenic potential of *Staphylococcus* spp. isolated from buffalo milk and the milking environment merits further study.

Epidemiology and Animal Economics

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Characterization of Staphylococcus spp. isolated from buffalo milk by Matrix Associated Laser Desorption-Ionization - Time of Flight Mass spectrometry (MALDI_TOF MS)

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There is growing demand for and production of water buffalo milk products in Brazil and other countries. Like cattle, water buffaloes are susceptible to mastitis; however, less is known about its aetiology or relatively lower incidence in these animals. Similar to cattle, more than 50 *Staphylococcus* spp. have been implicated as possible agents and coagulase negative staphylococci are thought to play an important role when primary mastitis pathogens are controlled. MALDI-TOF MS is becoming an increasingly common tool in the diagnostic laboratory. The aim of this study was to characterize staphylococci obtained from buffalo milk samples using MALDI-TOF. Milk samples (n=320) from 80 buffaloes were collected following physical examination and California mastitis testing (CMT). *Staphylococcus* spp. were isolated in large numbers and/or pure culture from 49 samples. Isolates were considered coagulase negative *Staphylococcus* spp. if they were gram-positive cocci, coagulase negative, and positive on genus specific staphylococcal PCR. These isolates were further characterized by MALDI-TOF MS. Of the 49 *Staphylococcus* spp. isolates obtained from the buffalo milk samples, 45 (91.8%) were characterized to the species level (score of ≥ 1.9) and 4 were identified to the genus level only with a score of ≥ 1.7 and ≤ 1.9. Similar to cattle, the most prevalent *Staphylococcus* spp. found in the milk was *S. chromogenes* (n=31). Others species found at lower frequency were: *S. caprae* (n=1), 1 *S. epidermidis* (n=1), *S. equorum* (n=2), *S. hominis* (n=1), *S. hyicus* (n=8), and *S. warneri* (n=1). In the hopes of understanding the lower prevalence of staphylococcal intramammary infections in water buffaloes, studies are currently underway to determine if these isolates carry the same of virulence genes as bovine isolates.

Epidemiology and Animal Economics

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The effect of weaning stress, sex and temperament on fecal microbiota in Brahman calves.

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A diverse microbial community, also known as microbiota, inhabit the mammalian digestive tract. The effect of weaning, sex classification and temperament on the bovine fecal microbiota is not fully understood. The goal of this study was to 1) investigate the effect of weaning on the fecal microbiota in Brahman calves, and 2) compare the fecal microbiota between males (bulls) and females (heifers) as well as between calm and temperamental animals at weaning (d0) and 4 days post weaning (d4). Temperament score (average of pen score and exit velocity) was used to classify calf temperament. Rectal grab fecal samples collected from the same 10 calm and 10 temperamental animals (5 males and 5 females of each temperament classification) at d0 and d4 were analyzed. Bacterial DNA was extracted using the PowerFecal kit (MoBio). Illumina sequencing of the V4-V6 region (*E. coli* position 530-1100) of the 16S rRNA was performed on a MiSeq. Raw sequence data were screened, trimmed, filtered, denoised and chimera depleted using default QIIME 1.8 software settings. Alpha and beta diversity measures were calculated and plotted. Differences in microbiota composition between sampling days, sex and temperament were assessed by unweighted and weighted UniFrac distance metrics and plotted on principal coordinate analysis (PCoA) plots. The ANOSIM function was used on UniFrac distance matrices to determine the statistical difference of microbiota between groups ($P<0.001$). Linear discriminant analysis effect size was used to determine differentially abundant bacterial taxa ($P<0.05$). The PcoA plot showed significant separation between the fecal samples from d0 and d4. A total of 51 bacterial taxa were differentially abundant between d0 and d4. Additionally, 23 taxa were differentially abundant between males and females at d4. Seven and 14 taxa were differentially abundant between temperamental and calm animals at d0 and d4, respectively. These data suggest that weaning has a major impact on microbiota composition in Brahman calves. Additionally, sex of the calf and temperament are associated with differentially abundant bacterial taxa within a sampling day.

Epidemiology and Animal Economics

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Investigation of Porcine Deltacoronavirus in Indiana swine population.

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Coronaviruses are enveloped RNA viruses that can cause disease in mammals and birds. Deltacoronavirus, the fourth genera of Coronaviruses, are the most recently discovered group of coronaviruses. One of the Deltacoronavirus species, HKU15, first discovered in Hong Kong, is named porcine deltacoronavirus (PDCoV). The virus was reported in the US swine population in April 2014, and became a reportable disease in June 2014. The virus is rapidly spreading throughout North America, currently affecting 15 states in the United States and Canada. The exact pathogenesis of PDCoV infection is yet unknown, but it is thought to cause similar clinical signs as porcine epidemic diarrhea virus (PEDV) and transmissible gastroenteritis virus (TGEV), both of which are also coronaviruses. In this research, PDCoV RNA was detected by a reverse transcriptase real-time PCR assay focused on a highly conserved region of the matrix protein. The objective of this research was to investigate PDCoV spread in Indiana counties from diarrheic pigs in 2014. Clinical signs associated with PDCoV positive cases were evaluated, and the presence of other enteric viral pathogens was determined in the same samples. Currently, 12.9% of tested cases were PDCoV positive, affecting 10 counties in Indiana. The clinical signs associated with PDCoV positive cases included diarrhea, enteritis, anorexia, collapse, and death. Additionally, we observed that about 30% of the PDCoV positive cases were co-infected with PEDV, and that the number of PDCoV viral copies was less in pigs with PEDV co-infection. These findings will guide further studies to investigate the epidemiology and pathogenesis of the PDCoV

infection in Indiana as a newly emerging disease as well as its association with other viruses detected in the same animal.

Epidemiology and Animal Economics

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Prevalence of Brucellosis in dairy cattle in South Korea with special emphasis on epidemiology, risk factors and control opportunities

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Brucellosis is one of thedominant causes of reproductive failure and reduced milk production in livestock.While dairy cattleand their farmer have been suffering from re-emerging issues of *Brucella* infectionbrucellosis in dairy cattle in South Koreahas not been commonly reported with only partialevidence on disease status in the country.The aim of this study is therefore to underline the prevalence and status of brucellosis in dairy cattleherds in South Korea.

The study was conductedin seven distinctly different regions between January 2014 and June 2015 and included from 3168 farms in 29 dairy cooperativesthat produced the commercially available milk. Of the 29milk cooperatives, particularly, eight displayed positive reactions in the milk tests for brucellosis, which were included 15 dairy farms in seven provinces of three regions.

Thesurvey of *Brucella* infection in dairy cattle in Korea under domesticated farming conditions showed a low prevalence [0.44%; 95% confidence interval (CI), 0.24-0.74] inthe milk ring testwhich is a more common screening test in dairy herds in Korea. The result correlated well with the milk-ELISA test(kappa, 0.799; CI, 0.627-0.972), which indicated an adequate sensitivity (90.91%; CI, 58.72-99.77) and specificity (99.87%; CI, 99.68-99.97). Of the 15 positive herds in milk tests, ten herds (66.67%) were positive in the individual serological tests(kappa, 0.909; CI, 0.783-1.000).The majority of positive cases were mainly caused by spreading from neighbour farms (45.5%) and by resurgence (40.9%).Large herds with more than fifty cattle were more likely to be positive compared to smaller herds.

Bovine brucellosis in dairy cattleappears to be still prevalent at low levels in the distinct regions of South Koreawhere are allowed to call for urgent biosecurity.Nevertheless,low prevalence of dairy brucellosis reflects the potential to ensure the smooth progress of the control strategies in the country; moreover it is ultimately important to maintain a constant monitoring, including regular prevalence survey, for eradication of brucellosis.

Epidemiology and Animal Economics

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Evolutionary analysis of highly pathogenic avian influenza H5N2 outbreak in Minnesota and neighboring states

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Highly pathogenic avian influenza (HPAI) H5N2 was first detected in United States in December 2014. In early March, first case was confirmed in midwest, Minnesota and disease spread to neighboring states. In midwest, the outbreaks occurred between March and June, 2015. We used next generation genome sequencing on primary samples from early and late phase of the outbreak to analyze viral evolution within outbreaks. Thirty samples were collected from turkeys (n=5), drinker (n=9), air (n=12) and environmental (n=4). Of these thirty samples, fourteen, six and ten samples were collected in early, mid and late of the course of outbreak, respectively. A total, twenty-three samples were successfully sequenced. Phylogenetic analysis showed that HPAI H5N2 is a reassortant between Asian strain HPAI H5N8 and North American strain avian influenza. Asian HPAI H5N8 strain is an ancestor of five gene segments; PB2, PA, HA5, M and NS and other three gene segments; PB1, NP and NA2 retrieved from North American strain of avian influenza. Time to most recent common ancestor analysis indicated that the reassortment likely occurred during 2012-2014.2. Moreover, the results indicate that PB1, NP and NA2 reassorted as HxN2 first and subsequently acquired other segments from Asian H5N8 lineage. Due to lack of sufficient information on database we cannot specify the ancestor of PB1, NP and NA2. The results supported that migratory wild birds play an important role of viral gene transfer to generate novel strains with enhanced virulence and transmissibility. Active surveillance is proposed as an important strategy to monitor emergence of novel strains.

Epidemiology and Animal Economics

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Study of population awareness, attitude, and behavior in relation with rabies in animals in Georgia

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Purpose:

Rabies is an endemic epizootic disease in Georgia. It is mainly observed in large cities and villages near scrub/forested areas. From 2006-2013, 898 cases of rabies was confirmed in animals. From 2011-2013, 98 cases occurred in the Samegrelo Zemo Svaneti region. The aim of this study was to assess the knowledge, attitudes, and practices of the population in Samegrelo Zemo Svaneti to rabies in animals.

Methods:

A cross-study (single-stage survey) method was used. A two-stage cluster sampling method was used for c-survey analysis. The proportional hazard was established using a cluster sampling method of data from the National Statistics Office. Participants who were at least 18 years old were sampled from the village. Google Earth maps were then used for randomization, showing a 95% confidence interval, 50% of the expected prevalence +/- 5% precision.

Results:

Four hundred and twenty individuals were interviewed. Of the people interviewed (six refused): 48% were male; the average age was 51 years; and 43% had 12 years or more of education. Objective knowledge of rabies was equal between urban and rural populations as well as those with and without animals. The prevalence rate ratio (PRR) was 1.0 (95% CI 0.8-1.2); 1.1 (95% CI 0.9-1.4); 1.0 (95% CI 0.8-1.3) respectively within these groups. A group (n=29) of the study participants were professionals (e.g., veterinarians, public health workers, herdsman, etc.) and were

1.3 times more knowledgeable than the general population; PRR=1.3 (95% CI 1.0-1.7) and were 1.7 times more likely to vaccinate their animals. Sixty five respondents visited a doctor and had 1.4-fold greater knowledge than subjects with no physician contact (PRR = 1.4 (95% CI 1.1-1.7)).

Conclusions:

The data shows a statistically significant association between professional groups and rabies awareness and the efficacy of animal vaccination. Creating public educational campaigns is suggested. The campaigns should focus on: the sources/transmission/outcome of rabies; the importance of post-exposure prophylaxis; as well as animal vaccination and certification of vaccination. The results of this study will contribute to planning rabies prevention measures in the future.

Epidemiology and Animal Economics

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Active surveillance of African swine fever in commercial swine herds in the Republic of Georgia in 2014.

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Purpose:

African swine fever (ASF) is one of the most serious diseases affecting pigs. It is caused by African swine fever virus (ASFV), a DNA virus within the Asfarviridae family. It can be transmitted by direct contact among pigs or by Ornithodorus ticks. In domestic pigs it can cause a mild disease, however, most infections are highly lethal. Currently, there are no vaccines against ASF. Since its introduction into the Republic of Georgia in 2007, ASFV has spread throughout the Caucasus region affecting swine production throughout. Risk factors contributing to its rapid spread are unknown. Since 2007, reemergence of ASF has occurred, suggesting it is endemic in these regions. The long-term goal of our research is to generate knowledge necessary to protect swine herds in Georgia from ASF by: estimating ASFV prevalence; on establishing ASFV association with potential reservoirs; and on ASF disease reduction/management. Research was conducted to establish a baseline prevalence of ASFV in 1) domestic swine, and 2) ticks collected from domestic pigs and pig environments; as well as 3) to represent spatiotemporal distribution of ASF and vector surveillance data collected from ImereTi, Kakheti, Guria, Samegrelo-Zemo Svaneti, Qvemo Qartli, Racha, and Mtckheta-Mtianeti regions in Georgia.

Methods:

During 2014, 1,231 samples (blood/sera) collected from domestic swine herds were tested for ASFV at LMA. A limited number of ticks were collected from those premises and morphologically identified at LMA as Argas spp. and not tested for ASFV. Antibody and antigen ELISA kits (Ingezim) were used for serological analysis; and real-time PCR kits were used for molecular analysis (Tetracore).

Results:

All blood and serum samples tested negative for ASFV via qPCR or ASF antibodies via ELISA despite sporadic outbreaks of ASF outside the sampled areas. This suggests that these domestic herds are not the source of the disease

Conclusions:

In light of this data, future efforts to identify sources for ASFV will be concentrated in areas where risk of ASF might be high, (e.g., international bordering regions of Georgia) where screening will focus mainly on feral and wild pig populations, backyard pig holdings, and ticks.

Epidemiology and Animal Economics

034p

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The consequences of active immunization of the sheep with the strain stern bacillus anthracis. dr.t. bajrovic1, a.celes2. 1university of veterinary medicine, department of microbiology with immunology and infectious diseases with epizootiology, sarajevo, bosnia-herzegovina.2 veterinary clinic, knezevo, republika srpska.

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After the vaccination of 3175 domestic sheep against anthrax, done in Nov. 2014 in the anthrax endemic region, due to the substantial flooding of the area, 2 to 3 days following the application, the illness and death of the animals occurred - final count was 573, in 5 of the 23 herds and in a relatively short amount of time. Along with the overall weakness and strong limping, the sheep's illness was characterized by the painful, purple-colored, edema axillary areas that were spreading toward chest and udder area, as well as numerous hard and/or soft lumps in their stomach and legs, which after 20 days, started to ulcerate with serous or puss-filled content. Although the antibiotic treatment (penicillin and tetracycline based) and symptomatic therapy were immediately administered, many sheep died (263) and aborted (310). Bacterial examination of the contents taken from the softer lumps established the presence of the Trueperella pyogenes bacteria.

The breadth and the character of the pathological state frequently resulting in sheep death, coincidentally occurring with the use of the Anthrax Sterne vaccine, could have been affected by multiple factors, amongst them being the vaccination of younger sheep with OCUREV® vaccine for prevention of brucellosis three weeks prior to the actual anthrax vaccination; possible uncleanliness of the vaccine preparation or later pregnancy as well as the antibiotic therapy which, on one hand, made sense due to the attempt of treating the diseased animals and which, on the other hand, didn't make sense since it contains contraindication after the application of the anthrax vaccine. Laboratory analyses that are occurring currently, will undoubtedly bring more clarity to the entire scope of the situation, but there is no doubt that in this case, the main issue has been the destructive nature of the vaccine resulting in significant economic losses.

Epidemiology and Animal Economics

035p

035p

Pilot extension program to control bovine leukemia virus in michigan dairy farms

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Enzootic bovine leukosis (EBL) is a contagious retroviral disease of cattle caused by bovine leukemia virus (BLV). Most farmers have no idea what level of BLV infection they have in their herds, and do not know how to use management procedures to reduce BLV prevalence. We conducted an Extension program in which producers paid less than half price for a BLV Profile (40 cows tested per herd) with a second BLV Profile to be repeated a year later. This program was publicized at 6 dairy Extension seminars, 6 presentations at DHIA and MMPA meetings, and in 15 articles in various Michigan dairy trade journals and newsletters. The Extension program included consultations for the dairy producers with Extension professionals regarding BLV management, and a final review regarding the effectiveness of attempted control efforts. Extension consultations were also provided to the herd veterinarians. Of the forty-two herds in the program, 5 herds had no BLV. The overall BLV prevalence was 37% and was higher in older cattle. Our statistical analysis focused on the association between BLV prevalence and the major BLV management risk factors. Participating producers have been pleased with the program and were eager to receive further consultation regarding BLV.

Epidemiology and Animal Economics

036p

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A national survey of bovine leukemia virus: preliminary descriptive epidemiology

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As of May 2015, 50 herds from Michigan, Minnesota, New York, Pennsylvania, and Wisconsin were enrolled in our research group's national study of bovine leukemia virus (BLV). BLV is a δ-retrovirus infecting in approximately 80% of U.S. dairy herds and 30% of U.S. dairy cattle. Previous work by our group and others has shown that infection leads to decreased milk production, impaired immune response, and decreased longevity among positive dairy cattle. Much of this work was part of a 2010 study of 113 Michigan dairy herds. The objective of the current nationwide study is to confirm our findings from Michigan on a broader scope.

Herds were enrolled via their DHIA organizations and a BLV herd profile (BHP) was calculated for each herd, as previously described, based on testing a sample of 40 cows by milk ELISA. The mean BHP was 41.6% (0.0-93.6%). The state average BHP ranged from 32.6% to 56.2%. One way ANOVA showed that the differences in average BHP among states were non-significant. Linear regression modeling BHP on herd size was significant (p=0.0067), however did a poor job of prediction when small herds were included in the model (adjusted R-square 0.1278). Removing herds under 200 head from the analysis resulted in a better fitting model with an adjusted R-square of 0.4126.

The overall mean BHP of 41.6% is consistent with our group's findings in Michigan and previous national surveys. There was no significant evidence of variation in BHP between states, however this may become significant as more herds are enrolled from additional states. Previous studies have consistently found an association between herd size and within herd BLV prevalence. Among the herds enrolled in this study, this association is present as well, however the association is stronger when small herds are excluded. This is likely due to the wide range of BHP for herds in the small size category. Five of the 23 herds in this category had BHPs of 0% - i.e. there were no positive cows found among those sampled. On the other hand, 7 of the 10 highest BHPs were reported in this size category. Management practices common to smaller herds may explain this apparent contradiction. These associations will be evaluated further in latter portions of this study.

Epidemiology and Animal Economics

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Field trial methodology to reduce transmission of bovine leukemia virus in Midwestern US dairy herds

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Bovine leukemia virus (BLV) is a contagious retrovirus that that can cause immune system impairment, persistent leukemia, and malignant lymphosarcoma. Recent studies demonstrate an association between BLV infection and decreased milk production and cow lifespan. BLV is estimated to be present in almost 90% of US dairy operations, with approximately 40% of dairy cattle infected. In contrast, more than 20 countries worldwide have eradicated the disease, raising concerns about future exports of US dairy cattle and products. Eradication and management programs in other countries have focused on test-and-segregate or test-and-cull strategies, an impractical solution for herds with high prevalence. We have initiated several field trials to investigate the feasibility of reducing BLV incidence or prevalence in dairy herds through changes in herd management: Trial 1 - using new hypodermic needles and sleeves, Trial 2 - feeding frozen or pasteurized colostrum to calves, Trial 3 - test-and-segregate (or cull) ELISA-positive cattle, Trial 4 - super shedder management using proviral load data to inform culling and segregation decisions. The structure and progress of these field trials will be summarized as of December 2015.

Pathobiology of Enteric and Foodborne Pathogens

038p

038p

Host specificity of Lactobacillus johnsonii isolated from commercial turkeys

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Purpose:

Minnesota is the top producer of turkeys in the US. Over the last decade MN flocks have shown substandard growth, even with the use of antibiotic growth promoters, resulting in millions of dollars in lost revenue. This condition has been termed Light Turkey Syndrome (LTS). Current probiotics used in turkeys were derived from chickens and have not been effective against LTS. We hypothesize that host specificity is the reason for the lack of efficacy. Our goal is to identify bacterial strains isolated from the turkey gut that will enhance growth. Genomic and phylogenetic analyses were used to identify the best bacterial strain candidates for enhancing growth and feed efficiency in turkeys.

Methods:

We surveyed two tom flocks, one from Iowa and one form MN, both of which exhibit above average bird weights compared to surrounding farms. Ilea were collected weekly from day-of-hatch to 12 weeks. Bacteria were isolated using both aerobic and anaerobic culturing conditions on media targeting Lactobacillus species. From this collection, we focused on Lactobacillus johnsonii, a microbial biomarker of turkey gut succession. Phylogenetic analyses of our L. johnsonii isolates and non-turkey reference genomes were performed to determine their evolutionary relatedness.

Results:

Over 1,200 bacterial isolates were collected from both flocks, 116 of which classified as L. johnsonii. 19 different clades of L. johnsonii were identified based on single nucleotide variant analysis. Turkey isolates were more closely related to each other than any other sequenced L. johnsonii from other animal hosts, indicating host specificity even between chickens and turkeys. Shared and unique gene subsets of each L. johnsonii clade were identified and their predicted functions were compared to reference L. johnsonii genomes to establish a turkey-specific core genome.

Conclusions:

This work leads to the hypothesis that probiotics derived from chickens that depend on strain colonization may not be as effective as probiotics developed from turkey-source bacteria. The genomic repertoire of turkey-specific L. johnsonii may include genes that aid in the success of turkey-specific probiotic approaches.

Pathobiology of Enteric and Foodborne Pathogens

039p

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Analysis of a comprehensive Himar1 transposon library of Mycobacterium avium subsp. paratuberculosis.

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Purpose: *Mycobacterium avium* subsp. *paratuberculosis* (MAP) is the etiologic agent of Johne's disease in ruminants and has been implicated as a cause of Crohn's disease in humans. The goal of this study was to define essential genes in MAP on a genome wide basis.

Methods: In this study we constructed a comprehensive *Himar1* transposon library consisting of five independent sets comprising a total of ca. 1 million mutants. MAP K-10 was transduced with φMycomarT7 phage carrying *Himar1* derived *MycomarT7* transposon. Kanamycin-resistant transductants were isolated on complete Middlebrook 7H9 medium after a 6-8 week incubation period at 37°C. DNA was isolated from each set and sheared randomly by ultra-sonication to obtain ca. 500 bp fragments. Transposon-chromosomal junctions were amplified by PCR and amplicons subjected to high throughput Illumina sequencing to locate the transposon insertion sites.

Results: Bioinformatic analysis of Illumina reads from all sets indicated that the transposon inserted in 31,483 (60.10%) out of 52,384 TA sites, corresponding to 93.1% of the genes containing at least one transposon insertion. Applying a 4-state Hidden Markov Model (DeJesus and Loerger BMC Bioinformatics 2013, 14:303), we identified 328 essential; 1,103 growth-defect; 2,603 nonessential and 258 as growth-advantage genes. These assignments covered 4,292 out of 4,350 total genes in MAP genome. As expected, essential genes identified match essential functions in DNA replication (*dnaE*), transcription (*rpoB*) translation (*rpmC, rpsL*) and essential metabolic pathways as those involved in lipid biosynthesis (*fabD2, fadE9*). Out of 31 *Himar1* and 8 Tn*5367* isolated individual mutants previously identified as carrying transposon insertions inactivating coding regions, none (except for MAP1432::Tn*5367*) were identified as essential in our analysis. These results confirm the robustness of our assignments.

Conclusions: This study defines for the first time gene essentiality in MAP on a whole genome basis. In addition, we identified growth-defect and growth-advantage genes whose inactivation limit or promote growth under the conditions tested.

Pathobiology of Enteric and Foodborne Pathogens

040p

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Targeting intracellular pathogenic bacteria with a kanamycin antibiotic peptide conjugate

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The therapeutic value of aminoglycoside antibiotics against intracellular bacteria is limited due to their inability to traverse the eukaryotic membrane. In an effort to address this issue, we have conjugated the antibiotic kanamycin to an unnatural proline rich peptide P14LRR. The P14LRR peptide is a potent non-membrane lytic, broad spectrum antimicrobial with efficient mammalian cell penetration. This approach would allow for kanamycin to enter mammalian cells as a peptide conjugate linked via tether that will breakdown within cells. We have demonstrated

that in the reducing environment the linker is cleaved to release free kanamycin with a half-life of 1.5 h. The antimicrobial activity of the Kan-P14LRR conjugate was evaluated with Gram positive and negative bacteria providing MIC value of 2 μM for both E. coli and S. aureus. We investigated the ability of the peptide conjugate to clear the intracellular pathogens Salmonella enteritidis and Brucella abortus within J774a.1 cells. After 9h treatment with Kan-P14LRR, intracellular Salmonella and Brucella were cleared by 95% and 85%, respectively. We also investigated the ability of the conjugate to clear Salmonella using an in vivo Caenorhabditis elegans model. After a 24h treatment, Kan-P14LRR was able to clear over 70% of Salmonella from the host.

Pathobiology of Enteric and Foodborne Pathogens

041p

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Immune response and protective efficacy of intranasal vaccination of subunit vaccines for Campylobacter control in broilers

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Vaccination of poultry against Campylobacter jejuni, the leading bacterial cause of human enteritis in the developed countries, is regarded as an attractive intervention strategy to protect food safety. However, vaccinations of chickens against C. jejuni have had only partial success, primarily due to ineffective vaccination regimen for inducing strong mucosal immune response. Our previous studies have demonstrated that the outer membrane protein CmeC is a feasible and promising candidate for immune intervention against Campylobacter. In this study, large quantities of recombinant CmeC protein and the CmeC-based DNA vaccine pCAGGS-CmeC were purified and then used for preparing chitosan microsphere (CM) nanoparticles, creating CM-encapsulated subunit vaccine CM-CmeC and CM-pCmeC, respectively. The 7-day-old broilers (20 per group) were intranasally immunized with PBS, CM, CM-CmeC, or CM-pCmeC. The chickens received booster immunization on day 21 and were then orally challenged with C. jejuni NCTC 11168 (105 CFU/bird) on day 35. Both serum and lavage samples were collected at different time points for measuring systemic and mucosal immune responses. Intranasal vaccination with CM-CmeC significantly elicited CmeC-specific serum IgG and mucosal IgA response. However, the CM-encapsulated DNA vaccine did not trigger significant immune response compared to that in the control groups. Intranasal vaccination of chickens with the CmeC-based subunit vaccines did not confer significant protective effect against C. jejuni colonization. This study showed that protein subunit vaccine is more effective than DNA subunit vaccine to induce immune response via intranasal vaccination route in chickens. The CmeC vaccination regimen should be further optimized to trigger sufficient protective immunity in poultry.

Pathobiology of Enteric and Foodborne Pathogens

042p

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Lectin binding profiles of primary and immortalized bovine intestinal epithelial cell cultures (BIECs) and infectivity of primary BIECs to different enteric viral pathogens

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Intestinal epithelial cells play important roles in the recognition of pathogens and microbial interaction through the expression of certain surface carbohydrate receptors. Lectins specifically bind to sugar molecules and can be effectively used for recognition of sugars on the cell surface. Lectins MALII and SNA are specific to sialic acid receptors that are necessary for the binding of many enteric pathogens. In this study, newly developed bovine intestinal epithelial cell line (BIEC-c4) was characterized for the expression of various surface sugar moieties using a set of 23 lectins and flow cytometry. Three enteric viruses: bovine rotavirus (BRV), bovine coronavirus (BCV) and bovine viral diarrhea virus (BVDV) were also studied for their ability to infect and initiate pathogenesis in BIEC-c4 cells. BIEC cell lines immortalized with simian virus 40 large T antigen (SV40), human telomerase reverse transcriptase (hTERT) and human papillomavirus (HPV) E6/E7 genes were compared with BIEC-c4 cells for their lectin binding profiles. Primary BIEC-c4 cells bind to most of the lectins indicating the expression of a large variety of sugars. All three immortalized BIECs also showed similar lectin profile as that of primary BIECs with some significant exceptions. Lectin binding profile of hTERT-BIEC was significantly different from that of the BIEC-c4 for nine lectins. Likewise, lectin binding profile of SV40-BIEC was significantly different for five lectins. However, for HPV-BIEC, only two lectins showed significant differences in their binding. Immunofluorescence assay using FITC conjugated monoclonal antibodies confirmed that rotavirus strain NCDV and BCV isolate 13690 were able to infect BIEC-c4 cells. No viral protein staining was observed in BIECs infected with BVDV; however, cytoplasmic vacuolization was observed 18 h post infection. This study overall demonstrated that immortalization of BIECs significantly changed the binding profile of many lectins. While BIEC-c4 cells expressed receptors necessary for attachment and entry of BRV and BCV, productive replication of virus did not occur as no viral protein could be detected after few passages of these viruses in BIEC-c4 cells.

Ecology and Management of Foodborne Agents

043p

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Knowledge, attitude and practices associated with Bovine Brucellosis risk and transmission among occupationally exposed individuals in Ibadan, Nigeria.

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Purpose: To assess the knowledge, attitude and practice about the transmission, prevention and control measures used by individuals with high level of contact with animals to protect themselves against brucellosis.

Methods: A cross sectional study was carried out from January to March, 2013. A total of 184 respondents including cattle traders, meat sellers, butchers, veterinarians and herd keepers in Ibadan, South western Nigeria were selected for this study. Data was collected through a structured

questionnaire and analyzed using SPSS for windows version 15. Means, Proportions, chi-square, Odds ratio and 95% CI were computed.
Results: Generally, the knowledge level of the cause and transmission of brucellosis among our respondents was average (Mean score 9.22(18)). Highest and lowest knowledge scores were shown by veterinary surgeons (12.22 ± 3.01) and meat vendors (7.48 ± 3.12) respectively. Furthermore, risk practices such as handling animals with open wounds, milking of animals, consumption of unpasteurized milk, handling of aborted animals and cohabiting with animals were found to be significantly associated with the knowledge of brucellosis transmission ($p = 0.0001$).
Conclusions: Livestock workers/handlers except veterinarians posses poor knowledge of brucellosis risk and transmission as a serious zoonosis in man. There is urgent need for more public health enlightenment programs among livestock workers in Ibadan, Nigeria.

Ecology and Management of Foodborne Agents

044p
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Assessment of antimicrobial activity of chlorine against most prevalent poultry-associated Salmonella serotypes in a chicken-meat-based model
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Purpose: Despite frequent use of various FDA approved sanitizers in poultry processing, significant proportion of marketed poultry meat in the US remains contaminated with some of the Most Prevalent Poultry-associated Salmonella seroTypes (MPPSTs). These include Typhimurium, Enteritidis, Kentucky, Heidelberg, Montevideo, Mbandaka, Senftenberg, Infantis, Hadar, Schwarzengrund, 4,[5],12:i: and Thompson. The objectives of this study were to (i) develop a food-based model that simulates carcass chilling; a major step in commercial carcass processing, and (ii) determine the differences in the susceptibility of different MPPSTs against chlorine; a widely used carcass sanitizer.
Methods: To simulate the environment within the immersion chilling tank, our experimental model included filter sterilized chicken meat extract (CME) obtained from frozen chicken carcasses mixed with ice-cold chlorinated water (40/50ppm) in different concentrations. This model was challenged with ~1x105 CFU of MPPST isolates and their survival was tested at 5, 30, 60 and 90 min post-inoculation. The pH, level of organic matter and concentration of free and total chlorine were measured.
Results: As the CME concentrations increased, the amount of total and free chlorine reduced, the pH of the chlorinated water increased and the survival of different MPPST isolates also increased. At 1% CME [145.56±19.31 mg/L of total organic carbon (TOC); 50.39±6.97 mg/L of total nitrogen (TN)] and 2% CME (TOC-297.64± 30.03 mg/L; TN-112.18±12.75mg/L), all MPPST isolates died within 30 min. At 3% CME (TOC- 500.45±58.60 mg/L; TN- 193.26±21.72 mg/L), all MPPST isolates survived until 90 min except S. Mbandaka, S. Kentucky and S. Heidelberg died within 30 min, 30 min and 60 min, respectively. At 4% CME (TOC- 660.92±66.76 mg/L; TN- 258.72±24.33 mg/L), S. Mbandaka and S. Heidelberg survived until 90 min but S. Kentucky died within 60 min. At 5% CME (TOC- 790.25±79.14 mg/L; TN- 309.84±28.64 mg/L), S. Kentucky survived until 90 min.
Conclusions: MPPST isolates differ in their susceptibility to chlorine. The level of CME contamination is an important contributing factor for survival of Salmonella against chlorine treatment.

Ecology and Management of Foodborne Agents

045p
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Population dynamics and antimicrobial resistome of the most prevalent poultry-associated Salmonella serotypes isolated from the US poultry
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Purpose: The objectives of this study were to (i) identify the most prevalent poultry-associated Salmonella serotypes (MPPSTs) that are consistently and most frequently isolated from poultry products in the US during 2002-2012, (ii) determine the correlation between annual prevalence of MPPSTs in the poultry products and the incidence of human illness reported by CDC, and (iii) determine the antimicrobial resistome of 610 isolates of MPPSTs from various poultry sources from the US.
Methods: USDA-FSIS and NARMS Salmonella surveillance data from 2002-2012 was used to determine the percent prevalence of different Salmonella serotypes in the US poultry (raw, ground and retail meat) and ranked to identify MPPSTs. A simple linear regression model was used to calculate correlation coefficient between the percent annual prevalence of each MPPST in meat and annual incidence of human illness reported by CDC for years 2002 to 2012. The resistome of 610 isolates of MPPSTs from the US poultry was determined against seven antibiotic classes.
Results and conclusions: The MPPSTs identified based on the average annual percent prevalence in poultry meat included Kentucky (4.22%), Enteritidis (1.65%) Heidelberg (1.60%), Typhimurium including its biphasic variant (1.58%), Montevideo (0.20%), Infantis (0.16%) Schwarzengrund (0.15%), Hadar (0.15%), Mbandaka (0.13%), Thompson (0.12%) and Senftenberg (0.04%). All MPPSTs except Kentucky were recently identified by CDC among the top 30 clinically relevant serotypes isolated from human illnesses in the US. The prevalence of Enteritidis ($R2 = 0.650$) and Heidelberg ($R2 = 0.899$) significantly ($P < 0.01$) correlated with the annual incidence of human illness due to these serotypes. Most of the isolates were pan-susceptible (52%) or displayed resistance to 1 (24.5%) or 2 (20.5%) antibiotic classes, whereas few isolates (3%) were resistant to ≥3 antibiotic classes. Multi-drug resistance (≥3 antibiotic classes) including resistance to third generation cephalosporin (2.4%) was primarily limited to Heidelberg and Kentucky. Nalidixic acid resistance (1%) was limited to two strains each of Typhimurium and Enteritidis, but none were resistant to ciprofloxacin.

Ecology and Management of Foodborne Agents

046p

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Antimicrobial resistance and molecular characterization of Salmonella Kentucky isolates from human and poultry

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Purpose: Salmonella Kentucky is the most predominant serotype isolated from poultry in the US. Human S. Kentucky infections in the US have climbed from an annual average of 63 cases (1997 to 2005) to 99 cases (2006 to 2012). Multi-drug resistance (mdr) among S. Kentucky has also become a significant public health concern. The objective of this study was to determine the antimicrobial resistance and compare genetic relatedness between poultry and clinical isolates of S. Kentucky.

Methods: A total of 140 isolates from US poultry and 29 isolates from patients with diarrhea were subtyped by XbaI-PFGE and tested for antimicrobial resistance by disc diffusion test and presence of Salmonella genomic island 1 (SGI-1) by PCR.

Results: Based on PFGE patterns, two major clusters (A and B) were identified at a similarity level of 50%. Cluster A comprised solely of clinical isolates (n=20) and cluster B comprised of all poultry and 9 clinical isolates. Majority (80%) of isolates from cluster A were resistant to >3 antibiotic classes including flouroquinolones whereas 20% were either pan-susceptible or resistant to only 1 antibiotic class. In contrast, majority of poultry isolates from cluster B were pan-susceptible (32%) or resistant to only 1 (8%) or 2 (56%) antibiotic classes whereas only 4% were resistant to ≥3 antibiotic classes. Similarly, clinical isolates in cluster B were either pan-susceptible (55%) or resistant to ≤3 (45%) antibiotic classes. Resistance to 3rd generation cephalosporin was limited to few (3.5%) poultry isolates only. The chromosomal attachment site for SGI-1 between the thdF and yidY genes was intact for all isolates in cluster B, suggesting that these isolates were SGI-1 negative. In contrast, the SGI-1 integration site was occupied for all isolates in cluster A with 11 isolates positive for both left and right junctions, 6 isolates positive for right junction only and 3 isolates negative for both junctions.

Conclusions: The clinical isolates within cluster B are genetically similar to US poultry isolates and are likely of domestic origin. In contrast, fluoroquinolone resistant, SGI-1 positive clinical isolates within cluster A are likely of international origin.

Ecology and Management of Foodborne Agents

047p

047p

The role of species richness in infectious disease studies: A preliminary inquiry based upon Coxiella burnetii

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Purpose:

Species richness is a measure of how many different species coexist within a community or ecosystem. In cases of infectious disease, high levels of species richness can indicate a greater variety of hosts available for infection by a pathogen. Coxiella burnetii, the causative agent of the zoonotic disease Q fever, has been identified in numerous species of animals. Although there appear to be species dominant strains of C. burnetii, it is possible that an increased availability of host species in a community could help to maintain C. burnetii in circulation among these hosts regardless of strain. The objective of this study was to examine the relationship between the level of domestic species richness on goat farms in Indiana and the presence of C. burnetii in the goats on the farm.

Methods:

Samples of milk, vaginal mucus and fecal samples were collected from 654 goat does on 95 farms and tested by real time polymerase chain reaction (PCR) for the IS1111 transposon of C. burnetii. Farms with at least 1 animal testing positive for C. burnetii from any of the three types of samples were defined as positive. Information was also collected about the presence of other species of domestic animals on the farm. Domestic species richness was calculated as the total number of domestic species (including goats) present on each farm. A logistic regression model was constructed to test whether the number of domestic species present affected the odds of a farm testing positive for the presence of C. burnetii DNA.

Results:

Although not statistically significant, the results of the regression model demonstrate a trend of increasing species richness on a farm resulting in a decreasing odds of detecting C. burnetii DNA in goats on the farm (p= 0.2).

Conclusions:

The identified trend contrasts with the idea that limiting the number of species on a farm may help to protect against disease transmission. Future studies incorporating the testing of all species present on farms for C. burnetii, including wildlife and people, are indicated to better characterize the role that species richness plays on the prevalence of the bacterium.

Ecology and Management of Foodborne Agents

048p

048p

Quantification of six major non-O157 *Escherichia coli* serogroups in cattle hide samples by spiral plating and multiplex quantitative PCR methods

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Purpose:

Shiga toxin-producing non-O157 *E. coli* of serogroups O26, O45, O103, O111, O121 and O145 are major foodborne pathogens. Cattle are a major reservoir and shed the organisms in the feces which leads to hide and carcass contamination at slaughter. Estimating the concentration of non-O157 *E. coli* serogroups on cattle hides is essential to assess the efficacy of various interventions employed to reduce the pathogen load on this matrix. The objective of our study was to utilize the spiral plating method and multiplex quantitative PCR (mqPCR) to quantify six non-O157

E. coli serogroups in cattle hide samples

Methods:

Hide samples (n=240) were collected from four major processing plants in June and July, 2015. Samples were collected by swabbing 2,730 cm^2 of the brisket area using sponges moistened with Butterfield's phosphate buffer. Samples were spiral plated onto modified Possè medium. Concentration (CFU/100 cm^2) was determined by counting chromogenic colonies using a counting grid and testing ten randomly picked colonies individually by a multiplex conventional PCR targeting six serogroups and four virulence genes. Concentration of each serogroup was determined based on the proportion of colonies positive for the serogroup. DNA extracted from hide samples was subjected to two mqPCR assays: Assay 1 targeting O26, O103, and O111; and Assay 2 - O45, O121, and O145.

Results:

Of the 240 hide samples, 67 (27.9%) were quantifiable by spiral plating method for one or more serogroups and concentration of the serogroups ranged from 1.1 to 3.9 log CFU/100 cm^2. *E. coli* O103 (15.4%) was the most common serogroup quantified followed by O26 (12.5%), O45 (4.2%) and O145 (0.4%). Based on mqPCR, 76 (31.7%) samples were quantifiable for one or more serogroups and concentrations of the serogroups ranged from 3.1- 4.9 log CFU/100 cm^2. *E. coli* O103 (16.7%) was the most common serogroup quantified by mqPCR, followed by O26 (13.8%), O121 (7.1%), O45 (3.3%) and O145 (2.1%).

Conclusions:

Although mqPCR detected more quantifiable hide samples, the advantage with the spiral plating method is that, in addition to determining concentrations of non-O157 *E. coli* serogroups, it can identify those that carry Shiga toxin genes.

Ecology and Management of Foodborne Agents

049p

049p

Comparison of the microbiological quality of fresh produce from seasonal farmer's markets and retail grocery stores in Ohio.

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The frequent use of antimicrobial drugs in veterinary medicine can results in the emergence and dissemination of antimicrobial resistance in a variety of animal populations. β-lactamases confer bacterial resistance to critically important antimicrobial drugs used in both human and veterinary medicine. Livestock are an important emergence reservoir for zoonotic food-borne transmission of resistant enteric bacteria including Salmonella spp. Our aim is to describe the role of fresh produce, which may have been fertilized with livestock feces, in the zoonotic food-borne transmission of antimicrobial resistant bacteria. Samples of leafy greens, tomatoes, and cucumbers were purchased each week from various local farmer's markets and grocery stores. These samples were placed in buffered peptone water (BPW) and inoculated onto spread plates for detection and quantification of coliform bacteria. An aliquot of the BPW was cultured for the presence of Salmonella. To test for the presence of β-lactamase-producing bacteria, samples were enriched in a nutrient broth 2 µg/ml cefotaxime, then inoculated onto 3 MacConkey agar containing Cefoxitin, Cefepime, or Meropenem. We sampled 93 farmer's markets and 67 grocery stores. There are 6 samples which produced isolates resistant to cefoxitin and cefotaxime antimicrobials, indicating the blaCMY phenotype. No cefepime or carbapenem resistant isolaes were recovered. The mean coliform count was 27 and 22 CFU per 100 µl BPW rinsate for farmer's markets and grocery stores, respectively. No Salmonella spp. were detected. Our results indicate that there is little difference in microbiological quality between farmer's market and grocery store produce measured by the presence of antimicrobial resistant enteric bacteria or coliform contamination.

Ecology and Management of Foodborne Agents

050p

050p

Validation of single and pooled manure drag swabs for the detection of Salmonella ser. Enteritidis in commercial poultry houses

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The official FDA method for sampling of commercial laying hen operations specifies individual testing of environmental drag swabs for detection of Salmonella enterica ssp. serovar Enteritidis (Salmonella Enteritidis). The FDA has also granted provisional acceptance for the National Poultry Improvement Plan's (NPIP) Salmonella Enteritidis isolation and identification methods for individual testing of environmental drag swabs. Substantial cost and resource savings could be achieved if environmental swabs could be tested in pools (broth from a combination of swabs) rather than singly. This study compared the single-swab reference (standard FDA) and alternative (NPIP) methods with each other and with two-swab pools, and 4-swab pools using both methods. Single and multi-laboratory testing of replicate manure drag swab sets (n =525 and 672, respectively) collected from a Salmonella Enteritidis free commercial poultry flock was performed by artificially contaminating swabs with either Salmonella Enteritidis phage type 4, 8, or 13a at one of two inoculation levels: low, x =2.5 CFU (range 2.5 - 2.7), medium, x=10.1 CFU (range 9.6-10.5). For each replicate, a single swab (inoculated), sets of two (one inoculated and one uninoculated), and sets of four swabs (one inoculated and three uninoculated), testing was conducted using the FDA or NPIP culture method. Detection of Salmonella Enteritidis was affected by method, phage type, and pool size. The pooled NPIP method was not significantly different from the FDA method for the detection of Salmonella Enteritidis in single drag swabs in commercial poultry laying houses, and was also more efficient and cost effective.

Ecology and Management of Foodborne Agents

051p

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A short review of policies and indications of antimicrobial drugs for food animals in the U.S.

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Purpose: The first objective was to review historical availability of antimicrobial drugs for use in food animals in the U.S., and public policy for

this use. The second objective was to review the current per label indications of antimicrobial drugs for these animals.
Methods: For the first objective, relevant entries in the U.S. Code of Federal Regulations and U.S. Code, and literature on history of the FDA activities were reviewed to produce the timelines of introduction of individual antimicrobial drug classes in use in food animals and of major developments in public policy for this use. For the second objective, from the USDA-sponsored Food Animal Residue Avoidance Database (FARAD), information on all antimicrobial drugs labelled for food animals in 1999-2014 was extracted and cross-checked against the FDA's Green Book. The data-table collated for each animal species and its given production scenario contained all FDA approved antimicrobial active ingredients for the scenario. For each ingredient, its antimicrobial class and subclass, and its formulations, indications, routes of administration, dosages, and combinatory usages were included. To summarize the data for each species and scenario, the numbers of ingredients of each class and subclass labelled for use for each purpose and route of administration were counted. The weights of cattle and pigs in 1940-2014 were extracted from the USDA's Economic Research Service database.
Results and Conclusions: The history of availability of antimicrobials for use in food animals and of public policy for this use in the U. S. was reviewed. Current antimicrobial drug indications for each food animal production scenario were classified by purpose and route of administration. Frequencies of indication of each antimicrobial drug class and subclass for individual purposes and routes were described. Combining this information with the historical weights of cattle and pigs produced, examples were derived that demonstrated that indexes of antimicrobial use standardized per unit of animal weight vs. per head can lead to different conclusions about the over-time trends in antimicrobial exposure of food animals.

Ecology and Management of Foodborne Agents

052p
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Seasonal Escherichia coli O157:H7 infection of cattle using standardized doses demonstrate factors extrinsic to the animal drive increased summertime colonization
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Higher summertime incidence of human infection by enterohemorrhagic Shiga toxin-producing Escherichia coli O157:H7 (EHEC O157) is associated with increased prevalence of EHEC O157 in cattle during summer months. The factors, extrinsic or intrinsic to cattle, affecting this seasonal increase in EHEC O157 carriage among cattle are not fully understood. A series of four experimental challenges 6 month apart were performed to identify these factors. For this Holstein steers (N=20) exposed to ambient environmental conditions were challenged with two standardized doses of 4-strain EHEC O157 inoculum in summer and winter at 6-month intervals in two years. The density and duration of recto-anal junction mucosa (RAJ) colonization with EHEC O157 was compared among season (winter vs summer), dose (109 CFU vs 107 CFU), and challenge route (oral vs rectal). RAJ EHEC O157 colonization density was significantly lower ($P = 0.016$) and duration was shorter ($P = 0.052$) after the summer month challenges when compared to winter challenges, a seasonal pattern opposite to that observed naturally. RAJ colonization was not significantly different between two challenge routes, indicating that passage through the gastrointestinal microbiome had no effect on it. RAJ EHEC O157 colonization in both seasons was significantly lower following low dose challenges compared to high dose challenges ($P < 0.001$). The data support the hypothesis that increased summertime EHEC O157 colonization results from increased seasonal oral exposure to this pathogen and that the cattle are not predisposed to increased EHEC O157 colonization during summer months, either due to intrinsic factors or indirectly due to gastrointestinal tract microbiome effects. Further studies understanding the factors responsible for increased seasonal oral exposure of cattle to EHEC O157 are warranted.

Immunology

053p
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Vitamin D3 increases nitric oxide production in Mycobacterium bovis-infected bovine macrophages.
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Vitamin D3 also called cholecalciferol is a liposoluble prohormone derived from cholesterol. It is responsible for regulating calcium homeostasis associated with the onset of lactation in cattle. However there have been studies that show that 1,25 (OH) 2 D3 has the ability to modulate immune function. The aim of this study was to evaluate the effect of 1,25(OH)2 D3 on nitric oxide production in bovine macrophages infected with Mycobacterium bovis. Macrophage monolayers from cattle donors were treated with 40ng/ml of 1, 25(OH)2 D3, and infected with virulent M. bovis AN5 (MOI 10:1). Total RNA was reverse transcribed and used to analyze the relative changes in gene expression of iNOS by real time PCR. Cell supernatants were collected and nitric oxide production was assessed. Vitamin D3 treatment increased iNOS gene expression and nitric oxide production in macrophages infected with M. bovis. Our results suggest that vitamin D3 is involved in the production of microbicidal mediators in bovine cells. This work was supported by project PAPIIT IN- 220615 and CONACYT CB-167488.

Immunology

054p
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Evaluation of humoral immune status in porcine epidemic diarrhea virus (PEDV) infected sows under field conditions
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Porcine epidemic diarrhea virus (PEDV) is an economically devastating enteric disease in the swine industry. The virus infects neonatal suckling

pigs, causes severe dehydration, and mortality rate is up to 100%. Currently, available vaccines are not completely effective and feedback methods utilizing PEDV infected material has variable success in preventing reinfection. Therefore, comprehensive information on the levels and duration of effector/memory IgA and IgG antibody secreting B cell response in the intestines and lymphoid organs of PEDV-infected sows, and their association with specific antibody levels in clinical samples such as plasma, oral fluid, and feces is important. Our goal was to quantify PEDV specific IgA and IgG B cell responses in sows at approximately 1 and 6 months post-infection in commercial swine herds, including parity one and higher sows. Our data indicated that PEDV specific IgA and IgG levels in the plasma and oral fluid (but not feces) samples could be used for disease diagnosis purpose. PEDV specific B cell response in the intestines and spleen of infected sows decline by 6 months, and associates with the antibody levels in the plasma and oral fluid samples, but the virus neutralization titers in plasma remains high beyond six months post-infection. In conclusion, in sows infected with PEDV the presence of effector/memory B cell response and strong virus neutralization titers in plasma up to 6 months post-infection, suggests their potential to protect sows from reinfection and provide maternal immunity to neonates, but challenge studies are required to confirm such responses.

Immunology

055p

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Hydrogen peroxide inactivation of PRRS virus for vaccine preparation

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Porcine reproductive and respiratory syndrome virus (PRRSV) is an economically important animal virus that causes reproductive failure and respiratory track illness in pigs. Current inactivated vaccines have low efficacy and/or complicated time-consuming production procedure requiring application of hazardous reactants such as formaldehyde or binary ethylenimine. Here, we studied the possibility of hydrogen peroxide (H2O2) as a suitable alternative for inactivated vaccine preparation. Inactivation procedure was performed by incubation of North American PRRSV strain NADC-20 solution with different concentrations of H2O2 in various environmental conditions and evaluated its virucidal efficacy at two time points. In-vitro studies with MARC-145 cells, inoculated with inactivated viral solutions, demonstrate successful inactivation of virus with the absence of cytopathic effect even at very low H2O2 concentration of 0.5% after 1 hour of inactivation procedure. Cell proliferation assay was performed to confirm the results from microscopic observations. It was also found that catalase from bovine liver is more suitable reagent for removal of residual H2O2 from viral solution than iron (III) chloride, because it maintains neutral pH and it is biocompatible with living cells. Our studies suggest that H2O2-inactivated virus can be a promising candidate for further in-vivo investigation to confirm its efficacy in creating adequate immune protection.

Immunology

056p

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Isolation and characterization of equine pulmonary dendritic cells obtained from lung tissue.

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Dendritic cells (DCs) are professional antigen-presenting cells and have shown to be critical for the defense against pathogens including herpesviruses. Monocyte-derived dendritic cells from blood have been isolated and used in in vitro experiments for decades. Much less has been described about pulmonary dendritic cells (pDCs), particularly in veterinary species. However, using DCs isolated from the respiratory tract may be advantageous in the case of respiratory pathogens including equine herpesviruses. Thus, the aim of the present study was to develop a protocol for isolation and cultivation of pDCs from equine lung tissues, and to compare the phenotypes of those with DCs isolated from blood.

Following euthanasia, equine lung tissues were collected, minced, and digested using collagenase and DNase. Tissues were then passed through 40µm cell strainers to discard any bulky tissue and centrifuged with Ficoll to get purified mononuclear cells. The cell suspension was subsequently plated for three hours in 6-well plates to remove the non-adherent cells and the remaining monocytes were incubated with GM-CSF and IL-4 for 7 days to get pDCs. Immunocytochemical staining was performed to determine the phenotypes of the resulting cells and compare them with DCs isolated from blood using previously described methods.

Both pDCs and blood DCs expressed MHC class I, MHC class II, and CD44. However, DCs isolated from blood tended to have higher expression of MHC class II and CD44 when compared to pDCs. Furthermore, several histiocyte-associated antigens including Bla36, CD172a, and CD163 were stained strongly positive in blood DCs. A comprehensive comparison between pDCs and DCs from blood was performed to investigate potential differences in immune functions between these two types of DCs.

In conclusion, we have developed a protocol for isolation of pDCs from equine lungs and confirmed that the pDCs are antigen-presenting cells. However, while pDCs and blood DCs show characteristics of typical antigen presenting cells, their immune function may differ.

Immunology

057p

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Early weaning stress in pigs alters postnatal enteric neuro-immune development, inducing long-term disease susceptibility.

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Purpose: The current study investigated the impact of piglet early weaning stress (EWS) on 1) long term gastrointestinal barrier, immune, and enteric nervous system function; and 2) susceptibility of the gastrointestinal system to psychological stressors post weaning.

Methods: Male and female piglets were weaned either early (EWS) or late (LW) and compared for differences in adult fecal score. Ileum was collected to measure intestinal permeability, secretion, and mast cell and nerve function at 8 and 23 weeks of age using Ussing chambers, immunohistochemistry, and mast cell mediator activity assays. For stress susceptibility studies, 8 week old EWS or LW weaned pigs were

subjected to mixing stress, and 3 hours later, ileum was harvested for measurement of intestinal permeability.
Results: Compared with LW pigs, EWS pigs exhibited chronic intermittent diarrhea that was observed throughout the post-weaning period and into sexual maturity. Occurring concurrently with the noted diarrhea, underlying pathologies due to early weaning included increased intestinal permeability, increased mast cell number, and increased mast cell activation, which were found to contribute to increase intestinal barrier permeability. Routine histopathology was unremarkable. At 23 weeks of age, EWS induced a persistence in the number of enteric nerves, which were of the cholinergic, pro-secretory phenotype. Enteric neuronal stimulation induced stronger intestinal secretion in EWS pigs compared with LW ileum. Early weaned pigs also had increased susceptibility to intestinal barrier defects following a subsequent psychosocial stress. The clinical presentation of chronic diarrhea, increased intestinal permeability, increased mast cell activation, and increase nerve-mediated intestinal secretion due to EWS was greater in adult females compared with adult males.
Conclusion: Early weaning causes lasting gastrointestinal defects which present clinically as chronic diarrhea and increased host susceptibility to subsequent stressors. The observed enteropathy appears to be mediated by mast cells and cholinergic nerves, with a heightened response in female pigs.

Immunology

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Predicting relatedness of PRRSv strains based on whole genome T cell epitope content
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Purpose:
We have developed an immunoinformatics tool to identify the best PRRSv vaccine to use for herd-specific PRRSv outbreaks. PRRSv (Porcine Reproductive and Respiratory virus) is an enormous economic burden to pork producers. Like many RNA viruses, PRRSv has considerable genetic and antigenic variability that has made the disease difficult to prevent with standard vaccines and an efficacious, broadly cross-protective formulation has yet to be developed. While methods for comparing existing vaccines to PRRSv strains have been informative, the 'whole gene' approach fails to estimate cross-reactivity because it does not consider the T cell epitopes that are presented to the immune system, and whether they are conserved between the vaccine and the challenge strain. For that reason, we developed an Epitope Content Comparison (EpiCC) tool to better define the degree of conservation between PRRSv vaccines and circulating strains. We propose to use this tool to identify the best vaccine to use for herd-specific PRRSv outbreaks.
Methods:
We have previously developed a set of Swine Leukocyte Antigen (SLA)-restricted epitope prediction tools (PigMatrix). We further modified this tool to define relatedness based on T cell epitope content. Using this new tool (EpiCC) we screened complete genomes from 20 PRRSv and three modified live virus (MLV) vaccines. We identified epitopes predicted to bind to common class I and class II SLA alleles. Epitopes were compared and an epitope-based relatedness score (EpiCC score) was calculated. A distance EpiCC score matrix was constructed and used to built an 'epi-phylogenetic tree' that depicts the relatedness between strains based on epitope content.
Results:
We observed epitope content variability across proteins and strains and differences between the whole-genome phylogeny and the EpiCC-based tree
Conclusions:
EpiCC provides an objective approach to aid pork producers in vaccine selection when a PRRSv strain is introduced into a herd, and to select viral epitopes for incorporation into a MLV vaccine.

Immunology

059p
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BVDV infection significantly compromise the bovine neutrophils activity
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Neutrophils phagocytize the invading micro-organisms and activate the innate as well as adaptive immune response. Virus affecting the neutrophil may significantly affect the immune system. In the current study, effect of bovine viral diarrhea virus (BVDV) infection on bovine neutrophil's viability, neutrophil's cell surface marker expression such as CD14, CD18 and L-selectin, neutrophil's migration and phagocytosis ability was investigated. The neutrophils were isolated from peripheral blood and confirmed morphologically and phenotypically. Isolated neutrophils were infected with homologues pair of BVDV [e.g. cytopathic (cp) BVDV1b-TGAC or non- cytopathic (ncp) BVDV1b-TGAN] at 3 M.O.I., while lipopolysaccharide (LPS), 10ng/ml was used as positive control. Neutrophils were examined for apoptosis and cell surface expression at 1 hr and 6 hrs post infection (PI). While neutrophil's migration and phagocytosis ability was measured at 1 hr PI with BVDV. Results showed that both biotypes of BVDV induced the apoptosis in neutrophils with reduced the neutrophils migration and phagocytosis ability ($p > 0.05$). The cp BVDV induced comparatively more apoptosis than its homologues ncp BVDV. Similarly, cp BVDV significantly reduced neutrophil's migration and phagocytosis ability than its homologues ncp BVDV ($p > 0.05$). Both biotypes down regulated the L-selectin and CD14 and upregulated the CD18 expression on neutrophils with course of infection. In conclusion, current study revealed that BVDV cause innate immunosuppression by reducing neutrophils viability, its surface marker expression as well as neutrophil's migration and phagocytosis ability. Study should be repeated with other BVDV biotypes before drawing any definite conclusion.

Immunology

060p

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Standardization of an indirect ELISA test from purified protein immunodominant extracts of Brucella canis

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Canine brucellosis, caused by *Brucella canis,* mainly affect the reproductive system in dogs, causing economic losses in kennels breeders; in addition, it also causes disease in humans but, to this date, is unknown its importance as a public health risk. In Mexico, as in other countries, the serological diagnostic tests have variable levels of sensitivity, specificity, and cross-reactivity with other gram-negative bacteria that are also causing agents of diseases in dogs. The rapid slide agglutination test (RSAT), which is currently being used in the country for serodiagnosis, showed low sensitivity (only 4 of 10 dogs diagnosed as positive). In order to have a definitive diagnosis is necessary to perform bacterial isolation; however, the isolation requires the bacteria to be in acute phase, and it showed to be unsuccessful in animals with chronic infection. In this perspective, the development of diagnostic tests, exhibiting greater sensitivity and specificity is important for early diagnosis and detection of zoonoses. In this study we used the *B. canis* RM6 / 66 strain, that is used for RSAT, and we purified by FPLC immunodominant fractions to standardize an indirect ELISA in order to increase sensitivity in the diagnosis. We sonicated the bacteria and performed an indirect ELISA with sera from experimentally infected dogs, diagnosed as positive for bacterial isolation. Results show that 230 nanograms of crude antigen are sufficient to obtain positive and negative with the sera in a dilution factor of 1:1000. Subsequently, the soluble fraction was submitted to chromatography in Q sepharose in FPLC. The chromatogram showed different immunodominant fractions, which were resolved by SDS-PAGE and immunoblotting. The results showed two immunodominant fractions with a molecular weight between 55 and 70 KDa; with these fractions we performed an ELISA test obtaining O.D similar values to crude antigen. Our results suggest that both proteins are candidates for *B. canis* immunodiagnosis, making it necessary to validate the test with clinical cases.

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Immunology

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Borrelia burgdorferi-induced IL-10 expression in C57BL/6 mice is mediated by cyclic-AMP and requires CD14-dependent p38-MAPK activation

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Severity of arthritis in murine model of Lyme disease is influenced, among other factors, by genotype of the mouse. In response to infection with Borrelia burgdorferi, C57BL/6 and C3H/HeN strains of mice develop mild and severe arthritis, respectively. Differential expression of immunosuppressive cytokine interleukin-10 (IL-10) has long been associated with the observed differences in disease severity in C57BL/6 and C3H/HeN mice, but the underlying mechanism of IL-10 regulation was not known. In this study, we provide a mechanism of IL-10 regulation in C57BL/6 and C3H/HeN mice. We show that bone marrow derived macrophages (MΦ) from CD14-/- C57BL/6 mice express significantly lower levels of IL-10 and higher TNF as compared to the wild-type C57BL/6 MΦ. Pretreatment with a p38 inhibitor resulted in a significant decrease in binding of transcriptional factors STAT3 and SP1 to the IL-10 promotor region and decreased IL-10 expression. We also show that C57BL/6 MΦ produce higher levels of cyclic-AMP (cAMP) that positively regulate IL-10 production and inhibition of proinflammatory mediators. Exogenous addition of cAMP or PGF2-inducing sodium butyrate to C3H/HeN mice increased their IL-10 levels and lowered the levels of proinflammatory molecules. Moreover, we show that human PBMCs and THP-1 cells produce elevated levels of IL-10 when exposed to exogenous cAMP. In conclusion, our study shows that the IL-10 expression in C57BL/6 MΦ is mediated by intrinsically higher levels of intracellular levels of cAMP, and requires CD14 and activation of p38-MAPK.

Immunology

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US-UK Collaborative Swine Immune Toolkit Initiative: Development of new immune reagents for swine health, vaccine and disease studies.

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Pigs are a major food animal species for the US, UK and the world. Current research efforts require a broad range of immune reagents, but those available for pigs are limited. Our goal is to generate priority reagents, based on international input, and pipeline them for marketing. The UK partner will focus on mucosal targets, including chemokine receptors and IgE. US efforts will be aimed at expression of soluble proteins and CD molecules, and production of monoclonal antibodies (mAbs). The team has identified best immunization and screening strategies, set up collaborations with commercial partners for protein expression and mAb production, and updated protocols to evaluate reagent specificity. Our objectives are: 1) Clone and express swine cytokines and chemokines, IgE, CD antigens and receptors; 2) Prepare panels of mAbs reactive with swine targets; 3) Use reagents produced to develop new assays for swine immune markers, e.g., multiplex assays, intercellular staining, etc.; and 4) Provide the veterinary community with new commercial reagents and techniques for their research efforts. New panels of mAbs reactive with IL-6, IL-17A, IL-17F, IFNβ and IFNγ are currently being evaluated as well as mAb reactive with cell subset markers, NKp36 (NCR3) and CD19. Tools and reagents generated by this project will undoubtedly advance swine immune, disease and biomedical research efforts.

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Immunology

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Monoclonal antibodies against the spike glycoprotein of porcine epidemic diarrhea virus

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Porcine epidemic diarrhea virus (PEDV), a member of the family Coronaviridae, is a large (~28 Kb) positive sense RNA virus that causes severe diarrhea, vomiting and dehydration in pigs. The PEDV genome contains seven open reading frames encoding two polyproteins (ORF1a and 1b), one accessory protein (ORF3), and four structural proteins (S, E, M and N). The spike (S) protein is the major surface glycoprotein involved in virus attachment and entry, thus, is also the main target of host immune responses. The goal of this study was to develop and characterize monoclonal antibodies (mAbs) directed against PEDV spike glycoprotein. For this, mice were immunized with sucrose purified/UV-inactivated PEDV strain CO13. After a series of four immunizations, splenic cells were fused with myeloma cells lines and hybridomas selected in the presence of hypoxanthine-aminopterin-thymidine (HAT) medium. Thirteen hybridoma clones producing mAbs against PEDV S were identified. All mAbs recognized full length PEDV S expressed by a heterologous vector in cell cultures in vitro. Additionally, these mAbs reacted against PEDV infected Vero cell cultures, indicating their ability to recognize native spike protein expressed in the context of virus infection. All mAbs are currently undergoing further characterization aiming at identifying immunogenic regions and/or epitopes within the spike glycoprotein. Characterization of immunodominant domains of the spike protein may provide important insights on PEDV infection biology and immunity.

Immunology

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Testing the effectiveness of combinational adjuvants for subunit vaccines

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Adjuvants play a crucial role in vaccine efficacy especially
in inactivated or subunit vaccines. Their immunostimulatory contributions
include chemical stabilization of vaccine antigens, improving vaccine delivery by the slow release of the antigens for greater immune responses and decreasing the dose of antigen in the vaccine to reduce costs. Our laboratory has developed a low-cost easy-to-use emulsion (OW-14) that induces long-lasting antibody responses in swine. We have shown that vaccination-induced antibody responses induced by OW-14-adjuvanted Swine Influenza Virus (SIV) and *Mycoplasma hyopneumoniae* (MH) vaccine were comparable or greater than those produced by commercially available Flusure (SIV) or Respisure (MH) vaccines. In the present study, we tested the potentiating effects of combining OW-14 with other adjuvants in mice. Our results show that OW-14 works synergistically with another potential adjuvant, Trichosanthin (TCS). TCS is from the root tuber of *Trichosanthes kirilowii* used in traditional Chinese medicine. The use of OW-14+TCS combination as adjuvant to antigens, including ovalbumin (OVA) and Classical Swine Fever Virus envelope glycoprotein E2, induced robust antibody responses even with a single immunization dose. In conclusion, the use of combination adjuvants can make animal vaccines more efficacious and cost-effective.

Immunology

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Mycobacterium bovis biomass and culture filtrate protein extracts induce bovine macrophage apoptosis.

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Mycobacterium bovis, the causative agent of bovine tuberculosis is able to induce bovine macrophage apoptosis. However, the identity of individual proteins associated to this event is not well known. In this work we proposed to generate Mycobacterium bovis culture filtrate and cell extract protein fractions to identify their competence to induce cell apoptosis. Mycobacterium bovis AN5 strain was inoculated in Sauton medium and incubated (37°C) in static conditions during 8 weeks. Bacterial growth was separated in biomass and culture medium. Biomass was sonicated and proteins were obtained from the soluble extract (SE). Culture medium was filtrated to obtain the culture filtrate extract (CFE) and proteins were precipitated with ammonium sulfate at 70 % saturation. Protein concentration was 4500 µg/ml (202.2 mg of total protein) and 200 µg/ml (7.6 mg of total protein) from biomass SE and CFE respectively, as estimated by the Lowry method. Protein fractionation from SE and CFE was accomplished by molecular weight exclusion chromatography. About 100 fractions in a range from 5 to 100 kDa were obtained in both cases. Total protein fractions from SE, CFE and 5 to 100kDa of ES induced bovine macrophage apoptosis as measured by TUNEL. Our results demonstrate that mycobacterial proteins induce apoptosis independently of their location. This work was supported by project PAPIIT IN-220415 and CONACYT CB-167488.

Immunology

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Matrix Protein 2 based vaccine protects against swine influenza H1N1virus infection

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Extracellular domain of Matrix protein 2 (M2e) of influenza virus is highly conserved among Influenza A subtypes and is considered a universal vaccine candidate against influenza. In this study, we evaluated the immunogenicity and protective ability of M2e against swine influenza virus (SIV) infection using gnotobiotic pigs. Four repeats of M2e gene were inserted into recombinant adenovector (Adeno-M2e) that could transiently express the protein. Expression of M2e protein by adenovector was confirmed by indirect immunofluorescence assay. Twenty-five animals were divided in to 5 groups: 1) Mock-vaccination group received, a heat labile toxin double mutant (dmLT) of Escherichia coli as an adjuvant and recombinant adenovector containing the lac-Z gene 2) Vaccinated with dmLT and Adeno-M2e and challenged with H1N1 SIV 3) Vaccinated with dmLT and Adeno-M2e and challenged with H3N2 SIV 4) Challenged with H1N1 SIV and 5) Challenged with H3N2 SIV. Vaccines were delivered intranasally on 9 d and 30 d. Animals were challenged with SIV on 44 d and euthanized 5 d post-challenge (DPC). We determined the nasal shedding of virus on 3 and 5 DPC, virus load in lungs on 5 DPC, and also scored gross lung lesions. Animals vaccinated with Adeno-M2e and challenged with H1N1 SIV showed lower virus titers in the nasal swab at both 3 and 5 DPC and in lungs (5 DPC) compared to non-vaccinated animals that were challenged with H1N1. However, there was no difference in virus titer in nasal swabs and lungs between vaccinated and non-vaccinated animals that were challenged with H3N2 SIV. Percentage of lung gross lesions were estimated and the animals that received Adeno-M2e vaccine and challenged with H1N1 had significantly lower score. We also quantified the mRNA expression of various inflammatory genes in lungs of these animals using qRT-PCR. Animals vaccinated with Adeno-M2e and challenged with H1N1 had significantly higher expression of Interferons α and β, Interleukins 1α and 1β, and Interleukin 6 compared with mock control animals. These results suggested that Adeno-M2e vaccine elicited immune response and protection against H1N1 SIV infection but failed to yield protection against H3N2 SIV infection.

Immunology

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Novel monoclonal antibodies against the outer membrane protein-31 of Brucella melitensis are potential diagnostic tools.

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Purpose:
Brucellosis is a severe zoonostic disease in north China, where nearly 200 millions of peoples live in the endemic areas. Detection of Brucella infection is an essential step to prevent or control the brucellosis in humans and animals.
Methods: Constructed the recombinant Omp31and immunized with BALB/c female mice to product monoclonal antibodies (mAbs).Several immunoassays were used to detect the diagnostic potential of omp31 and the mAbs to omp31.
Results:
A total of 22 monoclonal antibodies (mAbs) were produced against the outer membrane protein-31 (Omp31) of Brucella Melitensis, of which 11 (50%) were IgG2a, 5 (23%) IgG1 and 6 (27%) IgM isotypes. Of 22 mAbs reactive to Omp31 antigen of B. Melitensis, 13 bound to five linear epitopes of 16mer peptides, 7 reacted with semi-conformational and 2 with conformational epitopes, respectively.By cross-matching the different recognition epitopes and reactivity levels, five IgG (1 IgG1 7A3 and 4 IgG2a 5B1, 1C1, 5B3 and 5H3) and three IgM (2D2, 2B6 and 5F11) clones of mAbs presented high potential as diagnostic antibodies, of which four IgG and one IgM could recognize the highly conserved linear epitopes of Omp31 from B. Melitensis, B. ovis, B. suis and B. canis strains.
Conclusions:
The potent monoclonal antibodies obtained in this study may provide substantial help potentially for uses in detection of Brucella infections by various immunoassays.

Immunology

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Ovine MHC class I and class II DRB1 allele polymorphism associated with cellular immune response in vaccinated sheep with the attenuated Brucella melitensis.

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Purpose:
Animal vaccination has been considered the most effective strategy for preventing brucellosis. The major histocompatibility complex (MHC) has been shown significantly associated with specific immune response to the vaccine in humans and animals, but little is known regarding

association with the attenuated Brucella melitensis vaccine currently used in sheep and goats. .
Methods:Extracted total RNA from 11 sheep PBMCs. PCR primer set-1 Af1 and Dr2 was used for amplification of MHC-I genes from Ka, and semi-nested PCR primer were used for MHC-I genes from Cm. The MHC-II *DRB1* exon 2 gene was amplified by PCR from genomic DNA with two modified primers. Finally, Identical sequences obtained from three or more clones were considered representative of true ovine MHC allele.
Results:
In this study, according to the polymorphisms of full-length nucleotide sequences, 11 ovine MHC class I (MHC-I) and 16 ovine MHC class II (MHC-II) DRB1 exon 2 (DRB1) alleles were identified from 11 vaccinated sheep, which were genotyped into five groups of ovine MHC-I (A-E) and six groups of ovine MHC-II DRB1 exon 2 (a-f) alleles, respectively. MHC-I B alleles Ovar-N*01501, Ovar-N*01601, Ovar-N*01701 and Ovar-N*01801 were dominant in 3/4 (75%) Chinese merino sheep (Cm) (P=0.024), while A1 alleles Ovar-N*01301, Ovar-N*02101 and one B1 allele Ovar-N*01302 were prevalent in 5/7 (71%) Kazak sheep (Ka) (P=0.061). Ten of 11 sheep were identified as carrying 2 or 3 MHC-II DRB1 alleles, in which DRB1a allele was widely distributed in 9/11 (81.8%) sheep.Diversities of MHC-I and MHC-II DRB1 allele genes and functional molecules were characterized for associating with the discrepancies of immune reactivity between individual animals. The polymorphism of peptide binding site in α2 domain between the motifs 149KEGA152 within MHC-I B alleles (Cm) and 149AAGE152 within MHC-I A1 alleles (Ka) might be responsible for much higher level in Cm significantly than in Ka responding to peptide stimuli of B. melitensis vaccine
Conclusions:
These results demonstrate ovine MHC-I and MHC-II DRB1 allelic polymorphism was correlated with the reactivity of T-cell response to attenuated B. melitensis vaccine.

Immunology

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Characterization of immunological gene expression in the intestine of healthy calves
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Gastrointestinal (GI) disease is a significant cause of morbidity and economic loss in young livestock. Understanding the pathophysiological significance of each element of the gut health 'triad' (immune system, microbiome, epithelium) will be useful in designing novel strategies to improve GI health in growing animals. In this study, we characterized mucosal inflammatory gene expression (IGE) profiles at different biogeographical sites of healthy calf intestines during the first 3 weeks of life. Twelve calves from second parity cows on a single commercial dairy were removed from their dams immediately after birth and fed clean, high quality colostrum. The calves were fed milk replacer and health-monitored daily. A subset of calves (n=3) were euthanized at days 1, 3, 7, and 21, and mucosal tissue samples were taken from the duodenum, distal jejunum, ileum, and colon. Quantitative real time PCR analysis was performed to quantify the expression of interleukin 10, toll-like receptor (TLR) 2, 4, and 10, and tumor necrosis factor-α genes. The calves were clinically normal, and showed excellent feed intake and growth rates over the course of the experiment. There was a correspondingly low level of IGE in all calves, at all intestinal locations, and at all time points. The ileal mucosa exhibited a significantly higher pattern of IGE than all other sites ($p<0.05$), and TLR 4 had a three-fold higher expression than all other genes. As expected, the IGE profile of the intestine of this carefully managed and healthy calf group was subdued. The higher background of inflammatory activation in ileal mucosa could possibly be due to the relative abundance of Peyer's patches in this region. In future studies, we will compare the IGE profiles with the intestinal microbial communities and epithelial function of sick and healthy calves. Garnering an understanding of the connection between the immune system and microbial populations could lead to management solutions to GI health and reduce the use of antimicrobials.

Immunology

070p
070p
Comparison of serum PTX3 from shipping through sickness and recovery in cattle
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Infectious respiratory diseases of ruminant are a serious health and economic problem for U.S. agriculture. In cattle alone, bovine respiratory disease complex (BRDC) costs the feedlot industry approximately 1 billion dollars annually. The level of resistance or susceptibility an animal has to one of the bacterial or viral pathogens of BRDC can be at least partly linked to the competence of its innate immune response - that arm of immunity that is not dependent upon vaccination or prior exposure to infectious insult in order to respond. Pentraxin-related protein 3 (PTX3) acts as an acute phase protein in response to infection and inflammation, and blood levels are often associated with outcome and/or severity of disease. PTX3 plays a role in innate resistance to viral infections such as cytomegalovirus and influenza virus, as well as to chronic lung infections caused by P. aeruginosa and K. pneumonia. At this time, the role of PTX3 in BRDC is unknown. Our objectives were to determine if PTX3 concentrations in serum differed between sham- and mass-medicated treatment groups, and if it differed between animals presenting with BRDC and those that remained healthy throughout a 28 day trial regardless of antibiotic use. Sixty head of cattle were purchased at each of three sale barns located in MO, TN, and KY for a total of 180 animals on trial. Cattle were transported to a feeding facility in KS where they were randomly allocated within source to one of two treatments, mass-medication with gamithromycin (n = 90) or sham saline-injection treatment (n = 90). Blood samples were collected for plasma at Day 0 (at sale barn), Day 1 (at KS facility), Day 9, and Day 28. Cattle presenting with BRDC were also sampled for plasma at the time of diagnosis and 5 days later. The plasma samples were assayed for bovine PTX3 using a commercial

quantitative sandwich ELISA. Of the 180 cattle on trial, 28 presented with BRDC. These cases were matched with control (healthy) animals from the same treatment, pen, and sale barn. Preliminary data thus far indicates that PTX3 concentrations do not correlate with BRDC or treatment. Additional analysis is on-going.
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Immunology

071p
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Comparative analysis of signature genes in PRRSV-infected porcine monocyte-derived dendritic cells at differential activation statuses.
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Activation statuses of monocytic cells including monocytes, macrophages and dendritic cells (DCs) are critically important for antiviral immunity. In particular, some devastating viruses, including porcine reproductive and respiratory syndrome virus (PRRSV), are capable of directly infecting these cells to subvert host immunity. Monocyte-derived DCs (mDCs) are major target cells in PRRSV pathogenesis; however, the plasticity of mDCs in response to activation stimuli and PRRSV infection remains unstudied. In this study, we polarized mDCs using the framework established in macrophages, and applied genome-wide transcriptomic analysis to compare signature genes involved in mDCs activation and response to PRRSV infection. Porcine mDCs were polarized with mediators for 30 hours, then mock-infected, infected with PRRSV strain VR2332, or highly pathogenic strain (HP-PRRSV), for 5 h. Total RNA was extracted from the pooled cells of four replicates, and used to construct sequencing libraries for RNA-Seq procedures previously optimized. Comparisons were made between each polarized and unpolarized groups (i.e. mediator vs. PBS), and between PRRSV-infected and uninfected cells stimulated with the same mediator. The overall similarity between samples was assessed in heat map plots calculating the Euclidean distance between regularized log transformed data to allow equal contribution from all genes. Principal component analysis, Poisson distance and DESeq2 dispersion estimates emphasized variations in comparisons. Clustering of samples was by virus strain and then by mediator. We then asked which genes showed the most variability across all treatments as these are likely to be the genes that will provide resolution for clustering the samples. Many of the genes showing the most variability were related to cellular structure and innate immune response. The magnitude of differentially expressed gene profiles detected in HP-PRRSV rJXwn06 infected mDCs as compared to VR-2332 infected mDCs was consistent with the increased pathogenicity of the HP-PRRSV in vivo.

Respiratory Diseases

072p
072p
Utilization of a behavior score to detect bovine respiratory disease in preweaned group-housed calves
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Bovine respiratory disease (BRD) is one of the most common diseases affecting preweaned calves and this disease can be detrimental to calf performance and welfare. Group housing has unique challenges for disease detection and a group-level screening tool may improve the ability of farm staff to detect disease. The objective of this study was to develop a behavioral based screening tool to detect BRD in preweaned group-housed dairy calves. This cross-sectional study included the evaluation of preweaned group-housed calves (n = 206) on 4 farms in Wisconsin. Calves were given a score of 0 (normal) or 1 (abnormal) for each behavioral category: abnormal posture when lying or standing, isolation, lethargy, and the willingness to approach a stationary person (2 separate approach tests were performed and each was scored separately). All behavior categories were summed to obtain a total behavior score for each calf and total behavior scores ranged from 0 (normal) to 5 (severely abnormal). After the behavior scores were performed, all calves were examined for BRD, diarrhea, and umbilical infections. The highest sensitivity and specificity of the behavior score for disease identification occurred at behavior score cut points of 2 and 3. Therefore, scores of 2 or greater or scores of 3 or greater were considered a positive test result for BRD and were further examined. Outcomes of a positive or negative behavior score for cut points of 2 and 3 were analyzed using logistic models, controlling for calf age and farm. A cut point of 2 was not significant for BRD ($P > 0.05$). At a cut point of 3 or greater, calves with BRD were 4.2 (95% CI: 1.7 to 10) times as likely to have a positive behavior score, compared with calves without BRD ($P < 0.01$). The sensitivity of the behavior score for the identification of BRD was 48%, as compared to the farm staff sensitivity of 29%. This behavior-based screening tool offers an additional method of disease detection, which was more sensitive than methods currently used on study farms for the identification of calves with BRD.

Respiratory Diseases

073p
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Genetic diversity and antigenic characterization of Quebec influenza virus strains isolated from pigs
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Swine influenza viruses (SIV) are a major cause of respiratory disease in pigs. Fast mutations in the heamagglutinin (HA) gene cause continuous change on surface glycoproteins of influenzavirus resulting in immune escape from the neutralizing antibodies. Since 1998, three predominant subtypes of SIV (H1N1, H1N2 and H3N2) have been circulating in the Canadian swine population. The objective of this study is to understand the evolutionary dynamics of SIV that currently co-circulate in Quebec swine herds. In order to meet this objective, 26 SIV PCR positive clinical samples were selected from animals experiencing respiratory problems in 2010 to 2015. Viruses were isolated from 20 out of 26 samples and all gene segments were analyzed from isolated viruses.
Lung tissues, saliva, and nasal swabs were collected from pigs and viruses were isolated in MDCK cells or in embryonated eggs. For sequencing and molecular characterization, all eight genes of the viral genome were individually amplified by RT-PCR and were sequenced. Phylogenetic

analysis revealed the presence of two genotypes H3N2 and H1N1 among Quebec swine population.
The HA gene analyses indicated that 13 out of 20 viruses are similar to the North American triple-reassortant SIV (tr-H3N2) allowing the classification of these viruses into the H3 cluster IV. Within these SIV strains, multiple gene reassortments occurred between tr-H3N2 and the pandemic H1N1 (pH1N1) and resulted in apparition of six reassortants. The seven remaining SIV strains were found to belong to subtype sw-H1γ group. The viral genome analyses of those seven H1 SIV strains revealed four reassortants resulting from gene reassortment between tr-H3N2 and pH1N1.
The resulting epitope mapping of the HA1 protein revealed 4 aa substitutions at 4 positions in 2 antigenic sites. Analysis of antigenic sites of HA3 showed that proteins had 40 aa substitutions at 29 positions in 5 antigenic sites.
Characterization of the Quebec swine influenza viruses clearly indicates several reassortments with in Quebec SIV strains where the pool of genes belong to North American swine tr-H3N2 from cluster IV and the pH1N1 virus. This information is important for SIV surveillance and public health.

Respiratory Diseases

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Development of fully-automated DNA microarray-chips for multiplex detection of bovine pathogens
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Bovine
respiratory and enteric diseases (BRD and BED, respectively) are major causes of economic losses to the beef cattle industry worldwide. Current diagnostic tests for these multifactorial diseases are single pathogen tests and therefore time-consuming and expensive. Furthermore, most of these assays include multiple steps that demand a high level of operator technical competence. A fully-automated multiplex assay that is capable of simultaneously detecting all relevant pathogens in a single assay could provide an efficient and cost-effective solution to these problems. DNA microarray, when combined with multiplex PCR, is a powerful and a highly sensitive tool in detection and differentiation of multiple pathogens in a single sample. Here we describe development of two DNA microarray assay chips, one for rapid identification of BRD pathogens and the other for BED pathogens. The BRD multiplex assay specifically amplified targeted gene segments from five bovine respiratory viruses and four BRD-associated bacterial pathogens. The BED multiplex assay successfully amplified targeted genes from six enteric viral, four bacterial and three protozoan pathogens. A comprehensive panel of DNA capture probes was designed for each target, specificity was determined, and successfully validated on reverse dot blot filters that would be used in fully-automated DNA microarray chips in which nucleic acid extraction, PCR amplification and probe-based detection steps are integrated. The two bovine microarray chips will allow efficient use of samples and significantly reduce the cost, labour and time required for detection of multiple pathogens. Full integration and automation of the work flow will minimize cross-contamination, possible zoonotic risks associated with some of the pathogens and reduce the skill levels required of technicians conducting these technologically advanced assays.

Respiratory Diseases

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Fostera® PRRS vaccine helps provide heterologous cross-protection against a wide range of diverse Porcine Reproductive and Respiratory Syndrome viruses
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Porcine Reproductive and Respiratory Syndrome (PRRS) remains one of the most economically devastating diseases afflicting the global swine production industry, causing an estimated $664,000,000 annually in damages in the US alone, and billions of dollars globally. Because of the ever increasing genetic diversity in PRRS viruses, it may be unrealistic to expect commercial PRRS vaccines to consistently deliver “complete” protection against disease. To the customer, who generally lacks the ability to compare to a non-vaccinated control group, the benefits of vaccination are not obvious when a heterologous field strain results in some level of disease in a vaccinated herd. Thus, partial protection may be incorrectly characterized as lack of protection.
Fostera® PRRS is a modified-live PRRS vaccine, attenuated by a novel process consisting of passaging the virus on proprietary cell lines engineered to express the CD163 PRRSV receptor. In order to determine the degree of cross-protection provided by Fostera® PRRS against genetically diverse challenge viruses, multiple efficacy studies were conducted. Percent genetic distance from the Fostera® PRRS vaccine ranged from 3.8% (Genotype 2, Lineage 8) to 35.5% (Genotype 1). Challenge viruses included Highly-Pathogenic PRRS from Asia, which appears to be the most virulent of known PRRSV strains. In all cases, a measurable protective effect was afforded by vaccination, resulting in milder disease. In those studies that included body weights, vaccination with Fostera® PRRS resulted in average daily gains following challenge that were higher than non-vaccinated controls.

Respiratory Diseases

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Avian-origin H3N2 canine influenza virus in the United States

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Historically dogs have not been considered as a major host species of influenza A virus (IAV). Such perception was broken when canine influenza virus (CIV) similar to H3N8 IAV circulating in horses was reported in the US for the first time in 2004 in racing greyhounds in Florida. Since then, H3N8 CIV has been associated with canine respiratory disease in 30 states including Iowa. While H3N8 CIV was prevailing in the US, an avian-origin H3N2 IAV emerged in Korea in 2006-2007 and has been associated with respiratory disease in dogs and cats. Since then, H3N2 CIV has also been reported from China and Thailand. In spring of 2015, an H3N2 CIV was identified in an outbreak of respiratory disease involving over 1000 dogs in Chicago area and then was quickly spreading to Midwestern states including Iowa. The present study was conducted to isolate and molecularly characterize CIV from an outbreak of canine respiratory disease in Iowa. In April 2015, an outbreak of severe respiratory disease occurred in a kennel in Northwestern Iowa with a history of boarding a dog that has previously travelled to Chicago and then later among dogs at a local veterinary clinic which provided healthcare to those sick dogs from the kennel. Nasal swabs were collected and submitted to our laboratory for testing. The swabs tested positive for IAV but could not be subtyped by PCRs. Later the virus was subtyped as H3N2 IAV by sequencing. Partial HA and NA sequencing showed that viruses in the samples were identical. Virus isolation attempts using 9-11 days SPF embryonated chicken eggs yielded an avian-origin H3N2 CIV. Full-length sequencing and phylogenetic analyses revealed close relatedness of this CIV to A/canine/Illinois/12191/2015(H3N2), which was linked to Asian H3N2 canine and feline IAV isolates. Passive monitoring of CIV cases among regional diagnostic laboratories indicated wide spread of H3N2 CIV with a few hot spots in the US, justifying continuous nation-wide surveillance of CIV and development of effective intervention strategies.

Respiratory Diseases

077p

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Effect of Bovine Viral Diarrhea Virus infection of bovine bronchial epithelial cells on neutrophil extracellular trap formation

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Bovine Viral Diarrhea Virus (BVDV) is an important viral agent in the bovine respiratory disease complex. BVDV infection results in functional alterations in neutrophils and other cells involved in host defense. One cell type that has received limited investigation in response to BVDV infection is the bovine bronchial epithelial (BBE) cell. Our laboratory has initiated studies of the effects of BVDV infection on a BBE cell line on interactions with Mannheimia haemolytica and leukocytes involved in the host response against M. haemolytica infection. BBE cells were infected with cytopathic BVDV type 1 at a multiplicity of infection of 1. Three days post infection conditioned medium was collected, centrifuged at 1500 rpm for 5 min, aliquoted and stored at -80ºC. Bovine polymorphonuclear leukocytes (PMNs) were exposed to various concentrations of BBE cell conditioned medium for up to two hours. Extracellular DNA was quantified and evaluated by fluorescence microscopy as a measure of neutrophil extracellular trap (NET) formation. Preliminary results indicate that BVDV infection of BBE cell results in release of soluble factors that cause NET formation by bovine neutrophils in vitro. These findings may have relevance to the viral bacterial synergism that characterizes severe bovine respiratory disease.

Respiratory Diseases

078p

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Use of matrix-assisted laser desorption ionization time-of-flight mass spectrometry to identify reclassified species, Streptococcus parasuis

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Streptococcus suis is a Gram-positive coccus and common pathogen in swine farms and is associated with meningitis, arthritis, bronchopneumonia, and septicemia. Thirty-five serotypes of S. suis (serotypes 1-34 and serotype 1/2) have so far been described on the basis of their polysaccharide capsular antigens. However, in 2014 researchers proposed that S. suis serotypes 20, 22, and 26 should be reclassified as a novel species with the name Streptococcus parasuis. The reclassification was based in part on phylogenetic analysis of the gene encoding the recombination/repair protein (recN). In our laboratory, 5 strains of Streptococcus that had previously identified as Streptococcus suis with conventional identification methods and also identified as Streptococcus orisratti by matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI TOF MS) were tested using a described PCR method for detection of S. suis-specific recN. All 5 strains were PCR negative and subsequently added to a MALDI TOF MS user database. Afterwards 21 standard strains of S. suis isolates representing 19 different serotypes were identified using MALDI TOF MS using the manufacturer's database plus the S. parasuis user database additions. These isolates were also tested using the S. suis-specific recN PCR. S. parasuis was identified in 2 strains (serotypes 15, 22) and were also negative using S. suis-specific recN PCR. The remaining strains were identified as S. suis, however, 3 of these were negative by S. suis-specific recN PCR (serotypes 7, 22, 25). An additional 113 field isolates were tested by MALDI TOF MS and S. suis-specific recN PCR. Of these 15 were identified as S. parasuis by MALDI TOF MS and all were negative by S. suis-specific recN PCR . However of the 98 isolates identified as S. suis by MALDI TOF MS only 8 isolates were positive by S. suis-specific recN PCR. These results indicate that MALDI TOF MS may be useful to identify S. parasuis more consistently than a S. suis-specific recN PCR. They also indicate that S. parasuis may include serotypes other than what has been proposed.

Vector-Borne and Parasitic Diseases

079p

079p

Analysis of intra-host genetic diversity in Rift Valley fever virus infection of ruminants

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Purpose:

Rift Valley Fever (RVF) is an arthropod-borne zoonotic disease caused by RVF virus (RVFV) of family *Bunyaviridae*, genus *Phlebovirus* that affects ruminants and humans. The tripartite viral genome is composed of large (L), medium (M) and small (S) segments of single-stranded, negative-sense RNA. The high mutation rate of RNA viruses increases their ability to adapt to diverse selective pressures.

Methods:

To analyze the intra-host viral diversity, samples were collected at different time points post infection from sheep experimentally infected with RVFV strains Kenya 128b-15 and SA01-1322 isolated in 2006 and 2001, respectively. Full-length viral genomes were amplified by RT-PCR from the viral inocula and tissue samples (blood, liver and spleen) collected during peak viremia. For analysis of the terminal regions of the genome segments, viral RNA was circularized and the tandem region containing both the 5' and 3' ends of the viral genome was amplified by RT-PCR. The amplified products were subjected to deep sequencing on the Illumina Miseq platform. The viral sequences from the tissues were compared with viral inocula and the type of mutations along with the frequency (% of viral population) of quasispecies was analyzed.

Results:

The L segment of the Kenya strain showed 4-6 synonymous mutations (5-32%), the M segment showed 2-4 non-synonymous mutations (13-99.7%) and two synonymous mutations (7-25%) within the NSm/Gn/Gc genes and one synonymous mutation in the UTR, and the S segment showed two synonymous mutations one in the NSs gene (16%) and one in the N gene (43-50%). The L segment of the SA01 strain showed two synonymous mutations (12.4-98.6%) with one in the UTR (99.8%) and one non-synonymous mutation (98.6%), the M segment showed three synonymous mutations (99%) in the UTRs, two synonymous mutations (78-99%) in the NSm gene and one non-synonymous mutation in Gn gene (7.4%); the S segment showed two synonymous mutations (99%) one each in the NSs gene and UTR and two non-synonymous mutations (99%) in the NSs gene.

Conclusions:

Deep sequencing provides insights into the dynamics of variants within the host but the biological significance of these mutations warrants further examination.

Viral Pathogeneses

080p

080p

Porcine deltacoronavirus induces apoptosis in swine testicular and LLC porcine kidney cell lines in vitro but not in infected intestinal enterocytes in vivo

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We compared the mechanisms of porcine deltacoronavirus (PDCoV) induced death of infected enterocytes in vivo and in infected LLC porcine kidney (LLC-PK) and swine testicular (ST) cells in vitro. Four, 11- to 14-day-old gnotobiotic pigs were inoculated orally with 8.8-11.0 log10 genomic equivalents (GE) of US PDCoV strains OH-FD22 or OH-FD100 (n=3), or mock (n=1). We conducted histologic analysis for PDCoV lesions, immunofluorescence (IF) staining for PDCoV antigens, and TUNEL assay for the detection of apoptotic nuclear fragmentation in singly or serially cut tissue sections from the small and large intestines of the acutely infected or mock pigs. Similar comparative assays were done on LLC-PK and ST cells inoculated with the cell-adapted strain OH-FD22-P44 (passage 44) in cell culture medium with 10 µg/ml of trypsin and 1% pancreatin, respectively. At post-inoculation days 3-4, infected pigs showed severe watery diarrhea and/or vomiting and diffuse, severe atrophic enteritis, with mild to moderate vacuolar degeneration of the enterocytes lining the atrophied villous epithelium, consistent with necrotic cell death. By IF, PDCoV antigens were evident in villous or crypt epithelial cells with either vacuolated or morphologically normal cytoplasm. By in situ TUNEL assay, no PDCoV antigen-positive, small and large intestinal villous or crypt epithelial cells showed positive TUNEL staining. In contrast, by double IF and TUNEL staining, most of the TUNEL-positive signals were found in PDCoV antigen-positive LLC-PK and ST cells that also showed cytopathic effects, such as cell rounding, detachment and clumping in clusters. Thus, PDCoV does not induce apoptosis in vivo in infected intestinal enterocytes of gnotobiotic pigs, but it induces apoptosis in vitro in two infected cell lines of swine origin, LLC-PK and ST cells. Vacuolar degeneration and eventual death observed in PDCoV-infected intestinal villous epithelial cells is not due to apoptosis, but likely to necrosis as a result of the cytolytic effects of the virus.

Viral Pathogeneses

081p

081p

Stress-activated protein kinases are involved in porcine epidemic diarrhea virus infection

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Since various viruses manipulate host cell signal transduction pathways including the p38 MAPK and the JNK/SAPK, the present study was initiated to determine whether such signaling pathways play a role in PEDV replication. We found that PEDV infection activates the p38 MAPK and JNK1/2 pathways. Although p38 activation was more effectively mediated in PEDV-infected cells than JNK1/2 activation upon virus infection, the maximal induction of phosphorylated p38 and JNK1/2 was observed similarly at 12-24 h postinfection. Notably, UV-irradiated inactivated PEDV, which is capable of allowing viral attachment and internalization but incapable of pursuing viral gene expression, failed to trigger phosphorylation of both p38 MAPK and JNK1/2, suggesting that PEDV replication is responsible for their activation. The activation of

both kinases was markedly diminished in the presence of specific inhibitors, SB202190 for p38 and SP600125 for JNK1/2. Direct inhibition of p38 MAPK or JNK1/2 activation by each chemical inhibitor significantly suppressed PEDV replication by affecting viral RNA synthesis, viral protein expression, and progeny release. In addition, the inhibitory effect of SB202190 in PEDV replication was shown to be more remarkable than that of SP600125. We previously demonstrated that PEDV induces caspase-independent apoptosis through the activation of mitochondrial AIF to facilitate viral replication and pathogenesis. Next, it was investigated whether p38 and JNK1/2 activation is associated with the PEDV-induced mitochondrial AIF-mediated apoptosis pathway. Independent treatment with each inhibitor did not inhibit PEDV-induced apoptotic cell death. Furthermore, the mitochondrial-to-nuclear translocation of AIF, a specific hallmark of PEDV-triggered apoptosis, was verified in the presence of each inhibitor by confocal microscopy analysis. These results indicate that SAPKs are not involved in the apoptosis pathway during PEDV infection. Taken together, our data suggest that the p38 and JNK1/2 signaling pathways play an important role in post-entry steps of the PEDV life cycle and beneficially contribute to virus replication.

Viral Pathogeneses

082p

082p

Genetic analysis of a pathogenic Korean PEDV strain at different passage levels

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In late 2013, severe large-scale outbreaks of porcine epidemic diarrhea virus (PEDV) re-emerged in Korea and rapidly swept across the country, causing tremendous financial losses to producers and customers. Despite the availability of PEDV vaccines in the domestic market, their protective efficacy in the field is still being debated. The unsatisfied effectiveness of current vaccines appears to be due to antigenic and genetic differences between vaccine and field epidemic strains. Therefore, cell culture isolation of epidemic PEDV prevalent in the field is urgently needed to develop next generation vaccines. In the present study, one Korean PEDV strain, KOR/KNU-141112/2014, was successfully isolated and serially propagated in Vero cells for over 60 passages. The *in vitro* and *in vivo* characteristics of the Korean PEDV isolate were investigated. Virus production in cell culture was confirmed by cytopathology, immunofluorescence, and real-time RT-PCR. The infectious virus titers of the viruses during the first 60 passages ranged from 10^5 to 10^9 $TCID_{50}$ per ml. The inactivated KNU-141112 virus was found to mediate potent neutralizing antibody responses in immunized animals. KNU-141112 virus inoculation causes severe diarrhea and vomiting, fecal shedding, and acute atrophic enteritis in neonatal piglets, indicating that strain KNU-141112 is highly enteropathogenic in the natural host. In addition, the entire genomes or complete S genes of KNU-141112 viruses at selected cell culture passages were sequenced to assess the genetic stability and relatedness. Our genomic analyses indicated that the Korean isolate KNU-141112 is genetically stable during the first 60 passages in cell culture and is grouped within G2b lineage together with the recent emergent global strains.

Viral Pathogeneses

083p

083p

Mutations in the genome of a virulent porcine reproductive and respiratory syndrome virus nsp2 deletion strain associated with attenuation

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Porcine reproductive and respiratory syndrome virus (PRRSV) is the most common and world-widespread viral pathogen of swine. We have previously reported genomic sequences and pathogenicity of two type 2 Korean PRRSV strains belonging to the virulent lineage 1 family, which contain 3 discontinuous 111-1-19 amino acid deletions in nonstructural protein 2 (nsp2 111-1-19 DEL) compared to VR-2332. In the present study, a virulent type 2 Korean PRRSV nsp2 111-1-19 DEL strain, CA-2, was serially propagated in MARC-145 cells for up to 100 passages (CA-2-P100). To evaluate the *in vitro* immunity between parental CA-2 and cell-adapted CA-2-P100 viruses, we sought to explore alteration of inflammatory cytokine and chemokine expression in cultured porcine alveolar macrophage (PAM) cells infected with each virus using quantitative real-time RT-PCR. The expression levels of TNF-α and MCP-1 were significantly down-regulated in PAM cells during the course of CA-2-P100 infection. Animal inoculation studies were conducted to comparatively analyze the pathogenicity between parental and high-passage CA-2 viruses. The results demonstrated that the virulence of CA-2-P100 was decreased showing normal weight gain, body temperatures, and lung lesions comparable to control. Furthermore, cell-adapted CA-2-P100 infection resulted in declined and transient viremia kinetics, as well as delayed and low PRRSV-specific antibody responses in pigs. In addition, we determined the whole genome sequences of low to high-passage derivatives of CA-2. The nsp2 111-1-19 DEL pattern was conserved for 100 passages, whereas no other deletions or insertions arose during the cell adaptation process. However, CA-2-P100 contained 54 random nucleotide mutations that resulted in 25 aa changes throughout the genome, suggesting that these genetic drifts provide a possible molecular basis correlated with the cell-adapted characteristics *in vitro* and the attenuated phenotype *in vivo*. Altogether, our data indicate that the cell-attenuated CA-2-P100 strain is a promising candidate for developing a safe and effective live PRRSV vaccine.

Viral Pathogeneses

084p

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Proteolytic processing of porcine reproductive and respiratory syndrome virus replicase orf1a polyprotein

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The porcine reproductive and respiratory syndrome virus (PRRSV) replicase open reading frame 1a (ORF1a) polyprotein is predicted to be proteolytically processed by virus-encoded proteases. In this study, the proteolytic processing products and actual cleavage sites were identified by using protein microsequencing, recombinant vaccinia virus/T7 polymerase expression system, and site-directed mutagenesis. Protein microsequencing identified that the cleavage site between nsp1alpha/1beta, nsp1beta/nsp2, and nsp2/nsp3 was located at H180/S181,

G385/A386, and G1446/A1447 of type 1 PRRSV, respectively. Transient expression of nsp2-8, nsp3-8, nsp4-8, nsp5-8 in the recombinant vaccinia virus/T7 RNA polymerase system identified cleavage products of nsp2, nsp3, nsp4, nsp7(alpha+beta) and various cleavage intermediates. The result revealed the existence of two alternative proteolytic processing pathways depending on the presence or absence of nsp2. The identity of each cleavage product was further verified by site-directed mutagenesis analysis of individual cleavage site in nsp3-8 and nsp4-8. This study constitutes the first in-depth experimental analysis of PRRSV replicase processing, and demonstrated the significant conservation of replicase protein processing scheme within the arterivirus family.

Viral Pathogeneses

085p

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Potential infectivity of Torque Teno Sus 1 (TTSuV1) viruses for humans

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Torque Teno viruses (TTV) are ubiquitous DNA viruses which establish lifelong infections in several mammalian species including humans and swine. Recent epidemiological evidence suggests that swine TTVs (TTSuVs) are closely associated with the other viral causes of swine respiratory disease and can potentiate other infections. Therefore, it is important to determine whether TTSuVs can infect other species, especially humans. Previously, we had determined that human, swine, farm and pet animal sera were positive for human and swine TTV DNA by PCR. As the presence of DNA does not necessarily imply infectivity, in this study, we determined whether the presence of TTSuV DNA also correlated with sero-conversion in humans. Bacterially-expressed TTSuV1 open reading frame 2 (ORF2) protein was used to develop an ELISA for the detection of TTSuV1 antibodies. Forty human sera and twenty swine sera were tested using the ELISA. Sixteen of the 40 human sera tested showed optical density values above the cut-off, which was determined as the mean of the lowest quartile values plus two standard deviations. Seven of the 20 swine sera were similarly positive. We further transfected human PBMC's with cloned and circularized TTSuV1 whole genome DNA. Human PBMC's supported the replication of TTSuV1 in the three serial passages tested, based on detection with a TTSuV1- specific immunofluorescence assay. The proliferative capacity of the TTSuV1 infected human PBMC's in response to stimulation with a mitogen was diminished. While our findings support the possibility of human infections with TTSuV1, the implications for pathogenesis remain to be explored.

Viral Pathogeneses

086p

086p

Development of technology for expression of ruminants Bluetongue virus antigen VP7 in transgenic Nicotiana tabacum plants

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Bluetongue is a viral infectious disease of wild and domestic ruminants transmitted by bloodsicking insects of genus Culicoides. In Ukraine, more than 5 years Bluetongue is not officially registered. But in recent years, due to global warming Bluetongue area of distribution has expanded to the north (crossed parallel 50° north latitude) and outbreak of Bluetongue was recorded in Eastern Europe.

The aim of our research was to develop a biotechnological process for expression of Bluetongue virus-specific antigen VP7 in transgenic plants Nicotiana tabacum and assess its diagnostic potential in ELISA.

Construction of the plasmids and transformation of bacterial strains was performed according to standard protocols. Tobacco genetic transformation was carried out by joint aseptic cultivation tobacco leaves explants of Nicotiana tabacum variety of Petit Gavana with overnight agro bacterium culture containing vector pBIN-BTV.

Total yield of purified antigen was equal to 0.2 mg per 1 g of plant biomass. The purified antigen VP7 was analyzed in indirect ELISA using positive (n = 5) and negative (n = 5) sera in three repetitions. The positive signal exceeds negative at least 15 times (0.3/0.02), indicating potent immunological specificity of plant-derived VP7 antigen. Moreover, the standard deviation of results in repetitions does not exceed 10.0%, indicating the reproducibility of ELISA.

So we have showed immunological activity of recombinant plant derived group-specific antigen VP7 of Bluetongue virus. Using obtained VP7 recombinant protein as antigen in veterinary practice will conduct of development an ELISA serological diagnostic tools for monitoring and prevention of spread ruminant Bluetongue through Ukraine.

Viral Pathogeneses

087p

087p

Epidemiological situation of African swine fever in Ukraine in 2015

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African swine fever (Montgomery disease (ASF)) is one of the most dangerous viral diseases that infect swine and it is currently spreading in European countries. ASF infection requires an obligatory registration to the World Organization for Animal Health. ASF is characterized by high mortality and causes significant economic losses, due to the lack of vaccines.To study the epidemiological situation in Ukraine with African swine fever in 2015 and to forecast for the future epidemiological situation in Ukraine. We gathered official data on epidemiological investigations of ASF outbreaks in Ukraine using the report by the State Research Institute's laboratory diagnosis and veterinary and sanitary expertise.Currently, (as of 20.07.2015) ASF was confirmed in 11 cases in Ukraine(8 domestic pigs and 3 wild pigs), among which, 7 cases were found in farms, dead wild boars with ASF were found in 3 locations , and small pig farm was infected in one location. In 2012-2013, cases of ASF penetration were effectively controlled to prevent its spread to the east and south. The more stable social and economic situation and the relatively low number of both wild and domestic pigs which are kept by the population in the east also contributed to its success.

The present ASF penetration in the north has features of epizootic with slow, diffuse spread of the virus in the wildlife. The relatively high

population of wild boar, combined with a low human population epizootic supervision significantly more complicated to conduct in this part of the country. Additionally, widespread poaching and economic difficulties creates conditions for inconspicuous penetration of the disease into the domestic pig sector. That's why the latest cases of ASF are likely to be just the tip of the iceberg of an ASF epizootic among wild boars in Polissya (with the possible epicenter near the Chernobyl exclusion zone).
1. Despite the efforts of the Veterinary Medicine of Ukraine, ASF continues spreading in Ukraine in wild fauna as well as in domestic pigs.
2. The number of cases of ASF has increased in domestic pigs in comparison with previous years.

Viral Pathogeneses

088p
088p
Production and characterization of monoclonal antibodies against emerging swine pestivirus
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Atypical porcine pestivirus (APPV) has been reported to emerge in the US swine herd recently. To prevent its potential outbreak, specific diagnostic reagents and assays are urgently needed. In this study, we generated a panel of monoclonal antibodies (mAbs) against putative E2 glycoprotein of APPV. E2 antigen was expressed as the recombinant protein in E. coli expression system. Western blot result confirmed that the antigen was specifically recognized by field serum samples from APPV-infected pigs. Subsequently, BALB/c mice were immunized with the E2 antigen and splenocytes of hyperimmunized mice were extracted and fused with mouse NS-1 cells. Hybridoma cells were established under the HAT selecting medium. Specific hybridoma clones secreting E2-specific antibodies were initially screened by immunofluorescence assay and ELISA, and monoclones were obtained by subsequent single cell cloning. A total of five mAbs against APPV E2 were obtained. Cross-reactivity with other pestivirus E2 antigens, including BVDV E2 and CSFV E2, was not detected. These mAbs provide a powerful tool for development of rapid diagnostic assays for early detection of APPV infection.

Ecology and Management of Foodborne Agents

089p
089p
Methicillin-resistant *Staphylococcus aureus* in beef products in Sulaimaniyah City-Iraq
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Methicillin-resistant *Staphylococcus aureus* (MRSA) is widely distributed in hospitals around the world and is a pathogen frequently transmitted through animal products to humans causing a variety of disease. There is a strong relationship between disease development such as staphylococcus food poisoning and MRSA in animals, especially beef products. The aim of this current ongoing study is to evaluate the prevalence and epidemiology of MRSA in beef products in Sulaimaniyah, Iraq and to investigate the possible connection between food source contamination and human cases of MRSA in the region. Samples of beef products were collected and subjected to a coagulase test and antimicrobial susceptibility test. MRSA isolates were selected and investigated for the *mec* A gene. Also the prevalence of Panton-Valentine Leukocidin (PVL) gene was also analyzed using PCR and plused-field gel electrophoresis (PFGE) methods.
During using coagulase test MRSA will be characterized, and through antimicrobial test using MRSA isolates will be sensitive to Vancomycin. *mec* A and PVL proteins identification are by PFGE. *mec* A and PLV genes will be detect in MRSA strain by PCR.This will be the first study investigating MRSA colonization in beef meet in Sulaimanyah city by PCR. Moreover, it suggests a possible human source of contamination during meat processing. PCR testing is taking place currently, full results are expected in November 2015. Further research is needed to examine the evolution of MRSA over the time that lead to different clinical signs in human.

ORAL ABSTRACTS

Bacterial Pathogenesis

1

Re-evaluating the LD50 requirements in the codified potency testing of Leptospira in the United States

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Potency tests for leptospiral vaccines have historically been performed by the codified hamster vaccination-challenge assays described in Title 9, Code of Federal Regulations (9 CFR), Parts 113.101-113.104 in the United States. These regulatory tests are likely responsible for one-third of the reportable USDA Category D and E laboratory animals used in the country, and both government and biologics industry are committed to reducing the statistic. Specifically, vaccinates (n=10/serial), challenge controls (n=10), and back-titration hamsters (n=20) are inoculated with virulent *Leptospira* with a valid test requiring >8 challenge controls to succumb to disease and a LD50 between 10-10,000. The purpose of this work was to evaluate the risk associated with the removal of LD50 limits; thereby, eliminating back-titration hamsters from the *in vivo* potency assays. The impact on potency testing serogroups *Leptospira (L.) canicola, L. icterohaemorrhagiae, L. pomona,* and *L. grippotyphosa* was determined through 1) retrospective analysis of industry and CVB serial release data from July 2011 – April 2015 and 2) evaluation through vaccination-challenge assays. For the initial vaccination-challenge assays (n=3/serogroup), one group recieved potent bacterin (PB) and six groups received subpotent bacterin (SB1- SB6). PB and SB1 were challenged with a single dilution of *Leptospira* between 10-10,000 LD50. SB2-SB6 received serial dilutions of more concentrated challenge. Additional assays (n=5) were conducted for *L. pomona* and *L. grippotyphosa* in which the LD50 for SB2-SB6 challenge was determined. Based on the retrospective analysis and *in vivo* assays, 80% of the challenge controls succumbing to disease reasonably ensured the minimal LD50 was administered. Subpotent vaccines containing *L. canicola* or *L. icterohaemorrhagiae* were not at increased risk for release when challenged with >10,000 LD50, but potent bacterins were at risk of being deemed subpotent when challenged with >10,000 LD50. *L. pomona* or *L. grippotyphosa* infected hamsters had a non-linear response to increasing concentrations of challenge, confounding the data interpretation.

Bacterial Pathogenesis

2

Dynamic attachment of differentially expressed Actinobacillus suis adhesins to host extracellular matrix components

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The tonsils of the soft palate of swine provide a first line of defense against antigens entering the upper respiratory tract. Paradoxically, they are also important sites of colonization for many microbes, and in some cases, may provide pathogens a route of entry into the bloodstream. Despite their importance, little is known about how bacteria colonize the tonsils or the host cell receptors involved in these interactions. Therefore, the objective of this study was to characterize attachment of *Actinobacillus suis*, an opportunistic pathogen of swine, to extracellular matrix (ECM) components.

Our previous studies of *A. suis* showed that several adhesin genes are differentially expressed in exponential or stationary phases of growth. Isogenic mutants were generated of genes for the putative fibronectin-binding protein YbaV, a biofilm-associated pilin, Flp1, and the outer membrane protein OmpA. ECM attachment assays of the mutant and wild type (wt) strains were conducted by growing cultures to exponential or stationary phase in BHI and cells were enumerated by plate counting. The cultures were added to wells pre-coated with purified human ECM proteins and incubated at 37ºC + 5% CO_2 for 0, 15, 30, 45, 60, and 120 minutes post-attachment. The A_{595nm} of wells stained with crystal violet was measured and standardized to input CFU.

There was greater attachment of wt *A. suis* to ECM components during exponential growth relative to stationary phase. Attachment of the ΔYbaV mutant to collagen IV, fibronectin, and vitronectin was lower in exponential cultures relative to wt, consistent with expression studies of *ybaV*. Differential attachment over time was also observed for ΔYbaV in exponential cultures. Surprisingly, attachment of the ΔFlp1 mutant to collagen IV was lower in exponential cultures relative to wt, despite previous reports of no known host receptor for Flp1. Preliminary data for ΔOmpA suggest findings similar to ΔYbaV, including time effects.

Complementation studies to confirm these findings are currently underway. These studies should provide insight into how *A. suis* and other invasive pathogens of the upper respiratory tract interact with the host.

Bacterial Pathogenesis

3

Direct enrichment and de novo sequencing of Mycobacterium avium subspecies paratuberculosis from fecal samples of cattle for genomic epidemiology applications

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Johne's disease (JD), caused by Mycobacterium avium subsp. paratuberculosis (MAP), is characterized by progressive non-treatable diarrhea and weight loss during its clinical stages. In the US, the herd prevalence of infection on dairy farms is estimated at 91%, costing over $250 million in economic losses annually. To control transmission of MAP between herds, it is important to prevent the introduction of infected cattle from other infected herds. Despite years of research on JD, little is known about the contribution of genotype to pathogenicity. With next generation de novo DNA sequencing, it is possible to interrogate presence of multiple genotypes within a sample, without compromising information due to culture and PCR biases that lead to selection of cultivable strains and losses of low abundance strains when single target amplification methods are applied, respectively. This method has the advantage of identifying all subtypes within a single sample. The objective of this study was to develop an efficient way to detect and track MAP infection in cattle by genotype. To accomplish the purpose, we selected three MAP-infected commercial dairy herds to collect individual-animal fecal samples. The Veterinary Diagnostic Laboratory performed a direct RT-PCR test on DNA fecal samples for MAP detection. Samples with Cp value below 24 were considered high positive for MAP. These samples were selected for peptide-mediated magnetic separation (PMS) using two chemically synthesized peptides, aMp3 and aMptD, using established methods. This approach achieved 85 to 100% capture of MAP. After capture, Fecal DNA extraction was performed using QIAamp DNA Stool Mini Kit and de novo sequenced on the MiSeq platform (300cycles). A total of 23 samples have been sequenced to date. CLC Genomics Workbench was used to

analyze all samples and showed high quality values between 30-40. Single nucleotide polymorphisms were detected using CLC, comparing each genome to its associated reference genome (MAP4). With these results, we combine information from bacterial genomics with advanced epidemiological modeling to improve understanding of the transmission mechanism of this pathogen in dairy herds.

Bacterial Pathogenesis

4

Improved diagnostics of Mycobacterium tuberculosis complex infections in Minnesota white-tailed deer

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Testing for mycobacterial diseases is costly, time-consuming and burdensome. Zoonotic bovine tuberculosis (BTB), caused by *Mycobacterium bovis (M. bovis*), is a major concern in low and middle-income countries with significant implications for human and animal health. Outbreaks in low-disease prevalence regions like the United States lead to significant economic losses. We applied 3 mycobacterial specific biomarkers (MB1895c, MB2515c, and Pks5) that have been validated in cattle, primates and white-tailed deer (*Odocoileus virginianus*), using indirect ELISA. Yearling white tailed deer fawns were experimentally infected with *M. bovis* at the National Animal Disease Center (NADC) and blood serum samples were collected at day 0 (Pre-infection), Day-19, Day-48 and Day-60 post-infection. At each time point, samples from four animals were collected. Samples from contemporaneous controls were also tested for the biomarkers. Results show that *M. bovis* specific biomarkers can detect BTB infection as early as 48 days post-infection in experimentally infected deer. Validation of the results was performed using deer sera collected by the Minnesota Department of Natural Resources from 2007-2010 through targeted BTB disease surveillance efforts. In total, 384 samples were tested for the presence of the biomarkers. We determined signal to noise ratios for each biomarker and found that the overall BTB prevalence per year using biomarkers suggests that BTB in deer declined after 2009 but is possibly persistent at low levels as subclinical disease. New methods of testing for tuberculosis are an important factor in disease control and elimination. Biomarker based diagnostics offer an alternative way to quickly test for TB in *Mycobacterium tuberculosis* complex (MTC) infection in animals and humans.

Bacterial Pathogenesis

5

High throughput screening to identify quorum sensing inhibitors to enhance the control of avian pathogenic e. coli

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Colibacillosis caused by avian pathogenic E. coli (APEC) is an economically significant bacterial disease of poultry worldwide affecting chickens, turkeys and other avian species. The infection in poultry leads to airsacculitis, peritonitis, synovitis and colisepticemia, and is associated with high morbidity and mortality. Currently, APEC infection is controlled by a commercially available vaccine; however it is not very effective. There is a need for complimentary treatment to enhance the control of APEC infection in poultry. Small molecules represent novel therapeutics for the control of infectious diseases because of their specificity, stability, and can target specific virulence mechanisms without inducing resistance. Here we focused on high throughput screening of small molecules to identify novel compounds that inhibit APEC quorum sensing (QS) Autoinducer 2 (AI-2). The QS has been shown to be an important virulence mechanism in many bacteria including APEC. Using the Vibrio harveyi BB170 (AI-1+, AI-2-) bioluminescent indicator, we screened a preselected library of ~4,200 compounds for their ability to inhibit AI-2 production by APEC 078. For this purpose, APEC 078 was grown in the presence of 200 µM of each compounds in a 96 well plate; culture free supernatant was used to assess the AI-2 activity by measuring the bioluminescence from the indicator bacteria. Culture supernatants from the V. harveyi BB120 (AI-1+, AI-2+) and E. coli DH5α were used as controls. The majority of the compounds did not impact the growth of APEC and only 1.5% (62/4,200 compounds) had 20-100% growth inhibition. Screening supernatants identified 9 compounds that inhibited AI-2 activity of the APEC with at least 4 fold decrease in bioluminescence. Further in vitro and in vivo characterization of these potential leads would facilitate development of novel therapeutics to augment APEC control in poultry.

Bacterial Pathogenesis

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High-throughput screening to identify novel anti-campylobacter compounds using a pre-selected enriched small molecules library

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Campylobacter is a leading cause of foodborne bacterial gastroenteritis worldwide and infections can be fatal. The emergence of antibiotic-resistant Campylobacter spp. necessitates the development of new antimicrobials. We identified novel anti-Campylobacter small molecule inhibitors using a high throughput growth inhibition assay. To expedite screening, we made use of a “bioactive” library of 4,182 compounds that we have previously shown to be active against diverse microbes. Screening for growth inhibition of Campylobacter jejuni, identified 781 compounds that were either bactericidal or bacteriostatic at a concentration of 200 µM. Seventy nine of the bactericidal compounds were prioritized for secondary screening based on their physico-chemical properties. Based on the minimum inhibitory concentration against a diverse range of C. jejuni and a lack of effect on gut microbes, we selected 23 compounds. Twelve of these 23 compounds also possessed low cytotoxicity to human intestinal epithelial cells (Caco 2 cells). Next, these 12 compounds were evaluated these compounds for C. jejuni in vitro clearance, with 10 compounds having an anti-C. jejuni effect in Caco 2 cells with some effective even at 25 µM concentrations. No resistance was observed to any of these lead compounds when cultured with lethal or sub-lethal concentrations suggesting that C. jejuni is less likely to develop resistance to these compounds. These novel 12 compounds belong to five established antimicrobial chemical classes; piperazines, aryl amines, piperidines, sulfonamide and pyridazinone. Exploitation of analogues of these chemical classes may provide Campylobacter specific

drugs that can be applied in both human and animal medicine.

Bacterial Pathogenesis

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A genomics-enabled 'sexual hybridization' strategy directly identifies the genetic basis for emergent virulence caused by a rapidly expanding clone of Campylobacter jejuni

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A clone of Campylobacter jejuni (clone SA) has emerged in the last decade as the main agent of sheep abortion in the United States and been associated with other ruminant abortions and human gastroenteritis recently. Previous genomics analyses have identified candidate loci that could explain clone SA's virulence, but the causative genetic basis remains unknown. We leveraged the natural competence of C. jejuni, an animal abortion model, and next generation sequencing to directly identify a single gene, the ubiquitous major outer membrane protein encoded by the porA gene, as the cause of clone SA's abortifacient phenotype. Specific mutations in porA are necessary and sufficient for mediating abortion. These gain-of-function mutations are conserved among 61 SA clone isolates . Furthermore, traditional genomics analyses were unable to identify porA as the basis for disease, highlighting the value of the 'sexual genomics' approach. Our results focus future studies for prevention and treatment of epidemic ovine abortion in particular and cast perspective on the utility of commonly used, unbiased genomic studies in general.

Bacterial Pathogenesis

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Identification and characterization of virulence factors of staphylococcus aureus isolates from cases of bovine mastitis

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The overall aim of this project is to identify and characterize virulence factors of S. aureus that enable this bacterium to be the most prevalent and dominant mammary glands pathogen of dairy animals. A total of 58 S. aureus isolates from cases of bovine mastitis were screened genotypically for 16 known virulence factors by PCR and phenotypically for production of hemolysins and slime on blood and Congo red agar plates respectively. Of 58 strains 11 cause strong hemolysis of α-toxin type, 31 causes weak hemolysis of β-toxin type and 14 were non-hemolytic. Similarly, 10 of 58 strains were strong slime producers, 41 of 58 were medium producers, 5 were weak producers and 1 is non-slime producer. Based on frequency of isolation and number of virulence factors present in the strain, predominant strains (n=4) were selected and further evaluated by RNA-sequencing and quantitative real time PCR (qRT-PCR) after co-cultured with bovine mammary epithelial cells in vitro. From this analysis we identified some virulence associated genes that were differentially expressed when S. aureus co-cultured with bovine mammary epithelial cells. These genes may be responsible for initial colonization of bovine mammary glands and are good potential targets for development of protective vaccine against S. aureus mastitis.

Bacterial Pathogenesis

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Genetic mechanisms of Salmonella enterica serovar Typhimurium for overcoming host stressors

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Salmonella enterica serovar Typhimurium (S. Typhimurium) is a zoonotic non-typhodial Salmonella (NTS) that causes a self-limiting foodborne gastrointestinal diarrhea in healthy humans. However, NTS causes systematic bacteremia, meningitis, pneumonia, osteomyelitis, and occasionally death in immunocompromised people. To establish infection, S. Typhimurium has to overcome host innate defense mechanisms. Here, we have applied a high-throughput transposon sequencing (Tn-seq) method to unveil the genetic factors required for the growth or survival of S. Typhimurium under various host stressors simulated in vitro. A highly saturating Tn5 library of S. Typhimuruium 14028S (≥ 175, 000 unique insertions) was subjected to selection during growth in the presence of osmotic stress (3% NaCl) or oxidative stress (1mM H2O2) or survival in extreme acidic pH (30 min in pH 3) or starvation (12 days in PBS). We have identified 68 genes (13 intergenic regions) and 43 genes (8 intergenic regions) required for optimal growth in the presence of osmotic and oxidative stresses, respectively. Similarly, 135 genes (26 intergenic regions) were required for survival in extreme acid, while 281 genes (59 intergenic regions) were required for survival during starvation. Interestingly, 8 genes encoding F0F1-ATP synthase subunit proteins were conditionally required for all 4 conditions studied. F0F1-ATP synthase complex is required for the ATP production from ADP, and regulates pH homeostasis in bacteria at the expense of ATP. We will further characterize the genes of significance by construction and phenotypic evaluation of deletion mutants. Comprehensive understanding of the genetic factors required for S. Typhimurium to overcome host stressors can provide novel insights for development of effective strategies to combat against this pathogen.

Keywords: Salmonella, stress, Tn-seq, infection

Bacterial Pathogenesis

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Driving through the fog: Understanding type A *Clostridium perfringens* in enteric disease of animals

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Current understanding of the role of type A *Clostridium perfringens* is reminiscent of the understanding of *E. coli* in enteric disease in the early 1960s. This fog is compounded by confusion engendered by the archaic system for typing *C. perfringens* and, more recently, by adoption of a sensitive real-time PCR test that targets a gene found in all *C. perfringens*. The fog is not helped by a dearth of veterinary bacteriologists. Recent studies are revealing *C. perfringens* to be a flexible, adaptable and versatile enteric bacterium, both as a commensal and as a pathogen, with clonal lineages adapting to specific hosts. A major breakthrough has been in understanding some of the basis of virulence in avian necrotic enteritis strains, and particularly the role of the novel pore-forming toxin NetB. A more recent discovery has been of an additional pore-forming

toxin, NetF, associated with canine haemorrhagic gastroenteritis and foal necrotizing enteritis. Genome sequencing and understanding the structure of the *tcp*-positive large conjugative virulence plasmids, including how the organism can carry several distinct *tcp* plasmids, is helping to clear the fog. Clostridial abomasitis of calves is not well understood, and type A enteritis of young piglets is probably a non-disease but a wonderful diagnosis of convenience. This talk will review recent studies at Guelph on these five diseases.

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Our specific objectives were to examine the role of NetB in pathogenesis and the true place of alpha toxin (CPA), if any, in disease development.

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An engineered *netB* mutant was avirulent, while strains with *netB* caused NE. A large percentage of *netB*^{neg} strains were virulent, raising the question, "_Is NetB an obligate requirement for development of NE?"

Chicks were challenged with NetB^{neg} field isolates, JGS5622 (a *netB* mutant), and a NetB^{pos} strain. Two-thirds of netB^{neg} strains were virulent. The positive control was virulent and JGS5522 was also virulent. The mortality rate was higher among birds challenged with netB^{neg} strains (32% vs 25%). Average weight gain among those challenged with either genotype was 40-50 g, while negative controls gained an average of 160 g. Thus, NetB may be critical for development of NE in most strains, but apparently not all. Mechanisms of virulence of NetB^{neg} strains may be fundamentally different than those for NetB^{pos} strains.

CPA is an important virulence factor in myonecrosis, but it was suggested that it has no role in pathogenesis of NE. An engineered *cpa* mutant retains full virulence *in vivo*. However, recovered birds have high titer antiCPA antibodies and antiCPA immunity protects vs challenge. We confirmed the earlier findings, in that >90% of 14-day-old Cornish × Rock broiler chicks challenged with a *cpa* mutant developed lesions compatible with NE. However, CPA was detected in amounts ranging from 10 to >100 ng per g of gut contents and mucosa in birds inoculated with the *cpa* mutant, its wildtype parent strain, and our positive control. There was a direct relationship between lesion severity and amount of CPA detected (R = 0.89-0.99). Thus, testing mutant strains for virulence in conventional birds (with *C. perfringens* as normal flora) may have compromised the results.

Bacterial Pathogenesis

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Clostridium difficile infection - A One Health problem.

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Clostridium difficile is a pathogen that infect both humans and agriculturally important animals. C. difficile strains isolated from humans and animals are virtually indistinguishable. This talk will outline the genomic mechanisms that might have contributed to the increased number of infections in both humans and animals and the need for a "One Health" approach to control C. difficile infection. This talk is intended to be part of special session on Clostridial pathogens being organized on December 7th.

Bacterial Pathogenesis

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Carvacrol reduces *Clostridium difficile* virulence without inducing gut dysbiosis

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Purpose:

Clostridium difficile (CD) is a nosocomial pathogen causing a toxin-mediated enteric disease in humans. CD predominantly affects hospital inpatients undergoing prolonged antibiotic therapy, which results in enteric dysbiosis, leading to CD spore germination, pathogen colonization in the intestine and subsequent toxin production. Therapeutic agents that inhibit CD toxin production without causing enteric dysbiosis could improve the clinical outcome of CD infections. In addition, inhibition of sporulation in the gut could prevent CD transmission. This study investigated the efficacy of CR in reducing CD virulence in vitro and in vivo.

Method:

Three hypervirulent CD isolates (ATCC BAA 1870, 1053, 1805) were grown with or without the sub-inhibitory concentrations of CR, and the culture supernatant and the bacterial pellet were collected for total toxin quantitation, Vero cell cytotoxicity assay and gene expression studies. Total viable count and heat resistant spore count were determined at different intervals. In addition, the effect of CR on the germination and spore outgrowth was determined by standard germination assay. Finally, the effect of prophylactic supplementation of CR on CD pathogenesis was studied in vivo, inducing antibiotic associated CD infection in mice. The effect or CR on mouse gut microbiome was also analyzed using 16S rRNA sequencing.

Results:

Carvacrol substantially reduced CD toxin production, cytotoxicity on Vero cells and sporulation ($p<0.05$). In addition, CR down-regulated the genes involved in toxin and spore production ($p<0.05$). Although CR did not inhibit CD spore germination, it completely inhibited spore outgrowth. The antitoxigenic concentrations of CR did not inhibit the growth of beneficial gut bacteria. Carvacrol supplementation significantly improved the clinical outcome in CD infected mice with a favorable shift in the gut microbiome.

Bacterial Pathogenesis

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Biofilms, wounds, and chronic infections

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"Microbiologists have traditionally focused on free-floating bacteria growing in laboratory cultures; yet they have recently come to realize that in the natural world most bacteria aggregate as biofilms, a form in which they behave very differently. As a result, biofilms are now one of the hottest topics in microbiology." (Potera 1996)

Biofilms are highly organized multicellular communities encased in an extracellular polymeric substance (or EPS). Biofilms contribute to most infections in the body and treatments cost the U.S. health care system billions of dollars annually. They are perhaps the preferred organization of bacteria in nature, animals and humans. It wasn't until the early 1990s that the biofilm mode of growth was recognized as a relevant aspect of microbiology. Since then, the study of biofilms has expanded considerably, with the recognition that bacteria in a biofilm mediate many chronic human infections of the mucosae. Biofilms are notoriously recalcitrant to antibiotics and immunity, rendering them extremely challenging and costly to treat. This presentation will begin with an overview of the impact of biofilms on human and animal health. I will define the scope and spectrum of diseases involving bacterial biofilms. The mechanisms that mediate biofilm recalcitrance to antimicrobials and host immunity will also be discussed. Finally, in vitro and animal models that are used to study biofilm infections, as well as new and emerging therapeutic strategies are topics to be presented.

Bacterial Pathogenesis

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The Toll-like receptor 2 (TLR2) does not play a critical role during systemic and central nervous system infections by Streptococcus suis strains from different backgrounds

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Streptococcus suis serotype 2 is an important porcine bacterial pathogen and emerging zoonotic agent responsible for sepsis and meningitis. Sequence types (STs; determined by multilocus sequence typing) 1, 7, 25, and 28 are the most prevalent worldwide. The ST1 and ST7 have been described as highly virulent whereas ST25 strains present intermediate virulence. Meanwhile, North American (NA) ST28 are usually low-virulent whereas their Asian counterparts (AS) are highly virulent. Though exacerbated inflammation is a hallmark of the *S. suis* serotype 2 infection, the implicated receptors remain largely unknown. *In vitro*, the TLR2 has often been described as important. Nevertheless, its role *in vivo* has only been studied during the systemic infection using a ST1 and ST7 strain, where it was shown to play either an important or no role in the infection, respectively. In this study, the role of the TLR2 in both the systemic and central nervous system (CNS) infections caused by *S. suis* serotype 2 strains belonging to different STs was evaluated using TLR2 knock-out mice. The systemic (ST25 and ST28 strains) and CNS (ST1, ST7, ST25, and ST28 strains) infections were studied following intraperitoneal and intracisternal inoculation, respectively. Results showed that, during the systemic phase of the infection, TLR2 was not involved in mouse survival, in the control of blood bacteremia nor in systemic inflammation following infection with ST25 and NA ST28 strains, regardless of the dose used. Only a partial role was observed for the AS ST28 during the acute systemic inflammation, but without influence on mouse survival. Regarding the CNS infection, the TLR2 did not appear to play a major role in brain bacterial clearance or presence of clinical signs; its role in brain inflammation during meningitis is currently being determined. Globally, these results suggest that, with the exception of the previously published ST1 infection, TLR2 does not necessarily play a crucial role in *S. suis* serotype 2 infection for most strains from different backgrounds. Consequently, other TLRs and/or receptors could be implicated. As such, the role of MyD88, central to the TLR pathway, is under investigation.

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Interactions between Streptococcus suis and Haemophilus parasuis

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There is growing evidence that organisms which share the same niche can form synergistic and/or antagonistic relationships through among other things, metabolic interactions, chemical signals, physical associations, and immunomodulation. *Haemophilus parasuis* and *Streptococcus suis* are common commensals of the tonsils of the soft palate of swine that under certain conditions can also cause severe disease. Using representative strains serovars of *S. suis* and *H. parasuis* with high and low virulence potential, the interactions between these two important pathogens were studied in vitro. When cross-streaked against *S. suis* serovar 2 (SS2) on chocolate agar the growth of *H. parasuis* serovar 5 (HP5) and serovar 3 (HP3), but not other serovars was affected. In planktonic cultures, SS2 growth was enhanced in the presence of HP3 whereas as HP3 growth was reduced. We also found that *H. parasuis* and *S. suis* biofilm biomass was decreased in co-culture, reflecting a decrease in the relative biofilm cell numbers. These effects were demonstrated to be at least partly attributable to secreted factors. We could also demonstrate that HP5 protected SS2 from the effects of antibiotics in co-culture biofilms, while SS2 protected HP5 from complement proteins in swine serum. These results suggest *H. parasuis* and *S. suis* may increase the virulence of the other by preventing entrance into a quiescent biofilm form, as well as providing synergistic protection against antimicrobials and complement. A better understanding of the complex polymicrobial interactions between these and other residents of the tonsils of the soft palate in swine should help in the development of new approaches to reduce swine respiratory disease.

Bacterial Pathogenesis

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Biofilm formation by Mannheimia haemolytica on bovine bronchial epithelial cells.

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Mannheimia haemolytica is the most important bacterial agent associated with the bovine respiratory disease complex (BRDC). M. haemolytica cells initially colonize the tonsillar crypts in the upper respiratory tract of cattle, from which they can subsequently descend into the lungs to cause disease. Severe respiratory disease is often preceded by active viral infection of cattle. The mechanisms by which viral infections predispose the host to M. haemolytica are unclear. One possibility is that viral infection alters the structure of the epithelial lining to favor attachment and colonization by M. haemolytica cells. Our working hypothesis is that M. haemolytica colonizes the upper respiratory tract epithelium of cattle in the form of a biofilm. We have developed an epithelial cell monolayer system to study biofilm formation directly on host epithelial cells. Using fixed primary bovine bronchial epithelial cells, we observed M. haemolytica biofilm formation after a 48 hour incubation period, as assessed using with crystal violet staining. Biofilm formation was inhibited by adding the monosaccharide D (+) mannose, but not methyl α-glucoside, to the growth medium. M. haemolytica formed a co-biofilm with the related bacterial species Pasteurella multocida, which can also be involved in BRDC. We then investigated the effects of viral infection of the epithelial cells on subsequent biofilm formation by M. haemolytica. We observed limited effects of epithelial cell infection with bovine herpesvirus type 1, bovine viral diarrhea virus, or bovine respiratory syncytial virus on subsequent M. haemolytica biofilm formation. Further characterization of this model system will provide new insights into the potential role of biofilm formation by M. haemolytica in the pathogenesis of bovine respiratory disease.

Bacterial Pathogenesis

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Mannheimia haemolytica biofilm cells induce neutrophil extracellular trap (NET) formation in vitro

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Purpose: *Mannheimia haemolytica* is one of the causative agents of bovine respiratory disease in cattle and is a normal resident of the tosillar crypts. However, upon stress, the bacteria move down into the lungs where they can cause pleuropneumonia leading to variable mortality rates and high morbidity rates, which are estimated to cost North America 3 billion dollars of economic loss each year. Previous research has demonstrated that *M. haemolytica* can cause bovine neutrophils and macrophages to produced extracellular traps, which are web-like masses composed of chromatin, histones, and antimicrobial proteins that can trap and kill *M. haemolytica*. In this study, we investigated whether *M. haemolytica* biofilms trigger neutrophil extracellular trap (NET) formation.

Methods: *M. haemolytica* was grown overnight and then seeded into 24-well, tissue culture-treated plates or glass slides for various amounts of time. Biofilms were quantified by staining with crystal violet and absorbance measured in an automated plate reader. *M. haemolytica* viability was quantified by plating on sheep red blood cell agar plates. NET formation was triggered by incubating bovine neutrophils with logarithmically grown *M. haemolytica*. Scanning electron and confocal microscopy was used to examine NET formation and *M. haemolytica* cell aggregation.

Results: We demonstrate that *M. haemolytica* produces aggregates of bacterial cells that have similar properties to biofilms. These biofilm cells exhibit pleomorphic morphology which includes shortened and elongated rods. Addition of PMNs resulted in NET formation that trapped *M. haemolytica* cells and aggregates of cells.

Conclusion: These data indicate that adherent *M. haemolytica* produces pleomorphic cells, within biofilm-like structures, that induce NET formation by bovine neutrophils.

Biosafety and Biosecurity

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Characteristics of infection control practices at North American veterinary teaching hospitals

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Infection control is crucial in the operation of all veterinary hospitals, as those in the veterinary field are at an increased risk of occupational exposure to zoonotic diseases. In a 2008 study, 50% of Veterinary Teaching Hospitals ("VTHs") surveyed reported significant health problems due to zoonotic infections among hospital personnel. Cryptosporidium parvum infections accounted for 68% of these infections. The objective of this study is to characterize current infection control practices in place for the prevention of zoonotic disease infection, specifically infection of C. parvum, in VTHs.

All VTHs located in North America and that had been operational for at least one year were eligible to participate in the study. Phone surveys of biosecurity experts were conducted from July 2015- October 2015 and addressed policies for hygiene, surveillance, patient contact, education, awareness, and enteric infectious disease control.

Among participating VTHs, 50% reported significant health problems in personnel that most likely resulted from zoonotic infection. Of these, Cryptosporidium was identified as the most common agent. The majority of VTHs surveyed had a committee that oversees Biosecurity program activities as well as written Biosecurity policy documents, however only half conducted mandatory training on the biosecurity and infectious disease control program.

The results of this study will help to improve strategies for preventing zoonotic diseases, such as those associated with C. parvum, among veterinary personnel and allow for targeted educational tools to promote a safety culture in veterinary medicine.

Biosafety and Biosecurity

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Evaluating environmental sampling methods for detection of S. enterica in a large animal veterinary hospital

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Outbreaks of nosocomial salmonellosis in hospitalized animals have been responsible for the closure of multiple veterinary teaching hospitals resulting in significant financial loss and devastating health consequences for patients. Environmental surveillance for *S. enterica* can be used for early detection of contamination, which can help prevent the occurrence of outbreaks. Though there are several different surveillance methods currently in use in veterinary teaching hospitals, there have been few reports comparing their efficacy. The current protocol for environmental surveillance used at Purdue was put into practice in 2000 and incorporates the use of gauze sponges for environmental sampling. An alternative sampling method utilized in other veterinary teaching hospitals uses Swiffer® brand electrostatic wipes for environmental sample collection. For this study, it was hypothesized that use of Swiffer® wipes for sample collection would provide a more efficient environmental sampling method in terms of time, labor, and cost. A head-to-head comparison was performed in Purdue's large animal hospital with matched samples being collected using both the gauze sponge and Swiffer® collection techniques. Statistical analysis showed the sampling techniques have fair agreement (Kappa coefficient=0.3697) in terms of ability to detect *S. enterica* in the environment. However, the Swiffer® wipes required fewer workers, less time, and less physical effort to collect samples; a financial analysis also showed the Swiffer® technique to be more cost-effective. These results suggest that use of electrostatic wipes would be preferable to gauze sponges for routine environmental surveillance in the large animal hospital at Purdue University.

Biosafety and Biosecurity

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The importance of being clean: biosecurity measures in farm operations.

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Biosecurity measures have been developed to protect people, animals and plants against the transmission of infectious agents. The lack of these measures played an important role in several epidemics of highly contagious diseases. Farmed animals diseases such as porcine reproductive and respiratory syndrome, foot-and-mouth disease, and avian influenza, can be transmitted through fomites, objects or substances able to carry infectious organisms and, thus, spread an infection.

In farm settings much attention has been paid to live animal movements (i.e. direct contacts), considered as the major route of between-farm transmission. However, the spread through fomites has been crucial in many cases, such as during the 2001 FMD outbreak in the UK. In fact, this outbreak was stopped only after the imposition of strict biosecurity measures effective in preventing the contagion through fomites. This route of transmission, defined as indirect contacts, includes veterinarians, artificial inseminators, milk trucks, and animals transports among the most at-risk visitor categories. Between-farm indirect contacts have been poorly understood so far, because of easily available clear data.

The goal of this work is to evaluate the contribution of indirect contacts to a potential epidemic. We built a simple infection model to run simulations starting with one infected farm. Indirect contacts data were retrieved from a list of on-farm visits, and movement data were considered as well. We tested the contribution to spread of indirect and direct contacts, both separately and combined. We performed a sensitivity analysis on a parameter representing the relative effectiveness of the indirect contacts, compared to direct ones. The importance of this parameter lies in the fact that it could be "controlled" by enhancing the biosecurity measures, thus reducing the indirect contact effectiveness. We also tested the trade-off of directing control measures toward direct contacts, instead.

Results of this analysis could have crucial implications regarding the effects of biosecurity practices. In particular, these will provide important elements for decision-makers in veterinary health agencies.

Biosafety and Biosecurity

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Simultaneous detection of swine respiratory pathogens using a multiplexed detection assay

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Lawrence Livermore National Laboratory (LLNL), in collaboration with Kansas State University (KSU), has developed a multiplex molecular assay for rapid and sensitive diagnosis of respiratory diseases in pigs. The assay utilizes the x-Map technology developed by Luminex Corp. available currently in many diagnostic labs. The assay presented is well suited for high-throughput routine surveillance of multiple pathogens and is cheaper than multiple single-plex assays currently in use. The embedded foreign animal disease (FAD) pathogen beads can be readily removed for routine screening of domestic diseases, and re-introduced into the panel when there is an increased need for FAD surveillance. The newly developed assay can detect and differentiate 10 swine domestic and FAD pathogens, including four bacterial pathogens- Mycoplasma hyponeumoniae, Actinobacillus pleuropneumoniae, Haemophilus parasuis, and Streptococcus suis, and six viral pathogens- Porcine Reproductive and Respiratory Syndrome Virus (PRRSV), Swine Influenza Virus (SIV), Porcine circovirus type 2 (PCV2), Pseudorabies Virus (PRV), African Swine Fever Virus (ASFV) and Classical Swine Fever Virus (CSFV). All pathogens in the assay are represented by one to five unique DNA or RNA signatures for increased sensitivity.

The performance of each signature including sensitivity and specificity was assessed by testing against a panel of targets and near-neighbor agents. The sensitivity of the assay was determined using the target bacterial or viral nucleic acids, and purified plasmid constructs containing the specific target DNA fragments. The limit of detection (LOD) for PRRSV was 0.55 pfu/mL, PCV2 was 3.6 pfu/mL, and SIV was 0.55 pfu/mL.

Additional validation testing using experimentally infected and fielded samples is underway at KSU.
In summary, we have developed a sensitive and specific multiplexed assay for the simultaneous detection of two FADs and eight domestic swine pathogens. This respiratory disease panel is suitable for high-throughput surveillance and diagnostic testing of important domestic swine diseases and FADs.

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Rapid detection of foot-and-mouth disease virus using a field-deployable, reverse-transcription insulated-isothermal PCR assay

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Foot-and-mouth disease(FMD) is considered the most contagious infectious disease of mammals. It ischaracterized by vesicular lesions and ulcerations in the tongue, mouth andcoronary bands of infected animals. The clinical signs of FMD mimic a number of other viral diseases including vesicular stomatitis, bluetongue, swine vesicular disease and vesicular exanthema of swine, and therefore laboratory confirmation is essential. Current laboratory assays for foot-and-mouth disease virus (FMDV) detection include virus isolation, antigen ELISA, and molecularassays such as reverse-transcription polymerase-reaction (RT-PCR) and real-timeRT-PCR (rRT-PCR). These methods require the samples to beshipped to central laboratories, and highly skilled technicians and sophisticated instruments to perform the assays and interpret the results. In order to minimize the time span between sampling and results, simple, portable and rapid assays that can be performed on-site for detection of FMDV are highly desirable. Here we report validation of a highly-sensitive, *Taqman* probe-based, reverse transcription-insulated isothermal PCR (RT-iiPCR) assay for rapid detection of FMDV. The FMDV RT-iiPCR assay was performed on a user-friendly, portable and relatively inexpensive instrument. The assay targets the highly conserved FMDV 3D polymerase gene. It accurately identified 63 FMDV strains encompassing all seven FMDV serotypes, and showed no cross-reactivity with 19 clinically-relevant non-target viruses tested. The analytical sensitivity of the assay was comparable to that of our laboratory-based FMDV rRT-PCR assay. The clinical sensitivity of the FMDV RT-iiPCR assay was determined using total nucleic acid extracted from nasal and oral swabs, vesicular fluid, epithelial tissues and pen-based oral fluid samples (pigs) collected from FMDV infected cattle, sheep and pigs. The results demonstrated that the clinical sensitivity of the FMDV RT-iiPCR assay was comparable to that of the FMDV rRT-PCR assay. This novel, highly-sensitive and low-cost molecular assay could be deployed for rapidfield-based diagnosis of FMD.

Biosafety and Biosecurity

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Infectious disease transmission risks on livestock farms: biosecurity practices, dogs and education

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Dogs are often on livestock farms, where they serve important management and companion roles. However, they may also be involved in disease transmission between livestock and people. The objectives of this study were to evaluate dog ownership among Ohio livestock producers, dog husbandry and farm biosecurity practices, and determine the prevalence of *Salmonella* and ESBL producing organisms in the dogs.
A questionnaire inquiring about farm demographics, dog ownership and infection control/biosecurity practices was sent to a random sample of 2000 OH livestock farmers; respondents with dogs were asked to supply dog fecal samples. Fecal samples were screened for *Salmonella* spp. and ESBL-producing organisms ($bla_{\text{CMY-2}}$, $bla_{\text{CTX-M}}$, bla_{KPC} and $bla_{\text{NDM-1}}$).
The survey response rate was 47% with 446 livestock farm operators; 297 (67%) reported both livestock and dog ownership. Approximately 52% of households included at least one individual at higher risk to zoonotic disease. Dog-owning respondents frequently reported high-risk husbandry practices: 54% never leashed or fenced their dogs and 73% rarely/never pick up their dogs' feces. Dog and livestock contact was common (70%), of which 96% indicated at least one higher-risk management practice (e.g., direct access to sick livestock pens or new arrivals). Households with higher-risk members reported similar husbandry, biosecurity, and concern levels as compared to households without those members (all $P > 0.05$). Forty-eight respondents provided fecal samples from 67 dogs; 7% of the dogs were shedding *Salmonella* spp, 27% *E.coli* $bla_{\text{CMY-2}}$, and 6% *E.coli* $bla_{\text{CTX-M}}$. Feeding raw treats and received probiotics were significant risk factors for *E.coli* $bla_{\text{CMY-2}}$ carriage in dogs. Dog ownership and close dog-livestock contact were frequently observed. High-risk farm and dog practices were regularly reported and *Salmonella* spp. and ESBL-producing organisms identified in dogs, indicating a likely increased risk for disease transmission. The increased disease risk and a low level of disease concern suggest a need for improved education and outreach. Infection control/biosecurity training for farm operators and veterinarians is likely warranted.

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Development of an animal model for Schmallenberg virus

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Schmallenberg virus (SBV) emerged late 2011 in sheep and cattle in Germany associated mostly with mild transient disease; however, in naïve pregnant animals it causes abortion, still birth & congenital defects. SBV belongs to the Simbu serogroup virus of the family Bunyaviridae, genus orthobunyavirus and is transmitted by Culicoides. To establish an animal model for SBV, infection trials were conducted in cattle and sheep. In the first study, 10 sheep were assigned into two groups receiving either infectious cattle serum or cell culture-grown virus. In the second study, 9 calves were assigned into three treatment groups receiving either infectious serum, cell culture derived virus or infectious lamb brain homogenate. Differences in viral and serological responses between sheep and cattle during SBV experimental infection were assessed. RNA in the serum was first detected on day 2 or 3 post infection (dpi) in sheep and calves, respectively. RNAemia level peaked on 4 dpi in both species.

The highest RNAemia level in calves was 4 ct values lower than the highest level in the sheep. Animals inoculated with SBV infectious serum showed higher RNA levels compared to animals inoculated either with culture grown virus or the infectious lamb brain homogenate. Also, the onset of RNAemia in calves inoculated with the brain suspension was delayed by 3 days compared to the other two inoculum sources. SBV-specific antibodies in the serum of sheep and calves were detected by an in-house ELISA based on the recombinant nucleocapsid protein (NP). Sheep had a higher background reaction compared to cattle using the NP-ELISA. The bovine serum antibody response had a steady increase from 0 to 21 dpi, whereas in sheep a significant increase in antibody response was only seen at later time points post-infection. In conclusion, higher level of SBV RNAemia and SBV antibody responses make cattle a preferable animal model to study SBV infection, pathogenesis, and vaccine development. In addition, infectious serum and not cell-derived virus or brain homogenate seems to be the preferential material for challenge infection.

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Polymorphism analysis of prion protein gene in eleven pakistani goat breeds

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Current study was conducted in order to explore prions (Prp) protein gene in 89 animals belonging to 11 Pakistani goat breeds from all provinces of Pakistan. PCR amplification of 771 bp coding region of PrP gene was done and six polymorphic sites were observed which includes 126, 304, 379, 414, 428 and 718. The locus C.428 was found highly mutant in all breeds as compare to other 10 breeds. Beetal breed was observed more variable on this locus as compare to other breeds. On the basis of these PrP variants NJ phylogenetic tree was constructed through MEGA6.1 which showed that all goat breeds along with domestic sheep and Mauflon sheep appeared as in one clade and sharing its most recent common ancestors (MRCA) with deer species. Protein analysis has shown that these polymorphisms can leads to varied primary, secondary and tertiary structure of protein. Multidimensional scaling plot start with a matrix of item-item distances and then assign coordinates for each item in a low-dimensional space to represent the distances graphically which explained that all goat breeds are explicitly separated into different group as compare to other mammals, principle component analysis is orthogonal transformation to convert a set of observations of possibly correlated variables into a set of values of linearly uncorrelated variables similarly genetic distance which is a measure of the genetic divergence between species or between populations within a species. Populations with many similar genes have small genetic distances which indicated that how they are closely related and have a recent common ancestor.

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Burden and predictors of Staphylococcus spp. infections among dogs presented at a veterinary academic hospital in South Africa

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A six year retrospective study was performed to determine the burden and predictors of staphylococcal infections in dogs at a veterinary academic hospital in South Africa.

Records of 1,626 clinical samples submitted for diagnostic evaluation at the University of Pretoria Veterinary Academic Hospital were obtained, and 1,497 of which were from Gauteng province were selected for inclusion in the study. Descriptive analysis was performed to estimate the proportion of staphylococcal positive samples and multinomial logistic model used to identify predictors of infections with species of Staphylococcus (*S.aureus*, *S.pseudintermedius, S.epidermidis, undeferentiated staph spp. and negative isolate*) used as the out. Twenty six percent (95% confidence interval (CI): 24.2-28.8) of samples were *Staphylococcus* spp positive. Of these, 19% (CI: 17.0-21.1) were *S.pseudintermedius*, followed by *S.aureus* (3.81%, CI: 2.90-4.91) and *S.epidermidis* (0.73%, CI: 0.37-1.31). *S.felis* was isolated from one urine sample. A significant (p= 0.0047) difference in the distribution of isolates by age was observed. *S.pseudintermedius* was most frequent (4.3%) in 6-8 year old dogs while, *S.aureus* was more frequent in 4-6 year olds (1.1%). Both *S.pseudintermedius* (7.4%) and *S.aureus* (1.5%) were most frequently isolated from skin samples while S.epidermidis (0.23%) were more frequently isolated from urine samples ($p<0.0001$). The odds of *S.aureus* infection was 3 times (OR: 2.8, p=0.046) higher in dogs <2 years than those >8 years. Additionally, dogs 2-4 years old had higher odds (OR 4.3, p=0.0049) *S.aureus* infection than those dogs >8 years. Likewise, the odds of *S.aureus* infection was significantly higher (OR 5.2; $p = 0.008$) in dogs between 4-6 years old than those >8 years. The odds of infection with *S.pseudintermedius* was 2 times (OR 2.3; $p = 0.0002$) higher in 2-4 year dogs than those >8 years. Moreover, the odds of infection with *S.pseudintermedius* was also twice (OR 2.0; p=0.0011) as high in dogs 4-6 years old than in those >8 years. The study shows that the majority of the dogs presented carry *S. pseudintermedius* and that age is an important predictor of *S.aureus* and *S.pseudintermedius* in dogs.

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Saving Fido - Unearthing a novel topical antimicrobial for treatment of multidrug-resistant staphylococcal skin infections in companion animals

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Multidrug-resistant staphylococci, in particular methicillin-resistant Staphylococcus aureus (MRSA), are responsible for the vast majority of wound and tissue infections in the United States. In addition to MRSA, Staphylococcus pseudintermedius (in particular strains of methicillin-resistant Staphylococcus pseudintermedius (MRSP)) has been identified as an important emerging pathogen in animals and is a significant source of opportunistic skin infection in companion animals. Strains of MRSA and MRSP have been isolated that are resistant to several first-line antibiotics and drugs of last resort, such as clindamycin. This underscores the need for development of novel antimicrobials to deal with this emerging challenge in veterinary medicine. In the present study, we demonstrate the ability of a lead thiazole compound and five derivatives synthesized by our research group to inhibit growth of clinical isolates of MRSA and MRSP at submicrogram/mL concentrations in vitro. A time-

kill assay confirmed these compounds are bactericidal against both MRSA and MRSP. Derivatives of the lead compound exhibit an improved toxicity profile against keratinocytes (HaCaT) via a MTS assay. Furthermore, a microtiter dish biofilm formation assay revealed two compounds were superior to conventional antimicrobials in disrupting adherent staphylococcal biofilm (a problem that leads to recurrence of infection). The thiazole compounds retain their potent antimicrobial activity in vivo in a murine skin infection model, exhibiting similar activity to the commercial antibiotic mupirocin. Preliminary mechanism of action studies indicate these novel compounds may be targeting bacterial cell wall synthesis. Collectively, the present study demonstrates these novel thiazole compounds are promising candidates for development as topical antimicrobial agents for the treatment of multidrug-resistant staphylococcal skin infections in companion animals.

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A field-deployable POCKIT™ Nucleic Acid Detection System for specific and sensitive point-of-need detection of feline leukemia virus RNA and proviral DNA

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Feline leukemia virus (FeLV) is a naturally occurring gammaretrovirus of the domestic cat, causing malignant, proliferative and degenerative diseases of the hematopoietic system. Proviral integration is a significant step in retrovirus replication, resulting in regressive or progressive infection. Proper identification of the stage of infection is critical for clinical disease management and prevention of virus spread. Insulated isothermal polymerase chain reaction (iiPCR) with POCKIT™ system, a field-deployable device, is a specific and sensitive nucleic acid detection system aimed to help point-of care diagnosis of diseases. In this study, FeLV RT-iiPCR and iiPCR assays were developed for detection of a highly conserved region of the U3 LTR of FeLV to identify viral RNA and proviral DNA, respectively, in infected cats. Testing serial dilutions of templates, the detection limit 95% of the FeLV RT-iiPCR and iiPCR were determined by probit analysis to be 16 copies of RNA and 6 copies of plasmid DNA, respectively. FeLV RT-iiPCR and iiPCR detected serial dilutions of nucleic acids extracted from cells infected with the FeLV ATCC VR-717 strain and a FeLV-A clinical isolate with 10 fold less and equivalent sensitivity, respectively, compared to a previously published real-time PCR (qPCR). Both RT-iiPCR and iiPCR did not detect other feline pathogens including Mycoplasma haemofelis, feline coronavirus, feline herpesvirus, feline calicivirus, and feline immunodeficiency virus, demonstrating excellent target specificity. Finally, 85 clinical feline blood samples were tested by FeLV RT-iiPCR, iiPCR and reference qPCR in parallel. Compared to the reference qPCR, FeLV RT-iiPCR and iiPCR on POCKIT™ had excellent sensitivity (100% and 97%, respectively) and specificity (both 100%). The agreement between RT-iiPCR and qPCR was 100% (kappa = 1.0) and between iiPCR and qPCR was 99% (kappa = 0.975). In conclusion, FeLV RT-iiPCR and iiPCR on POCKIT™ could serve as an easy field-deployable tool for rapid, specific and sensitive point-of-need detection of both progressive and regressive FeLV infection, respectively.

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Temporal trends of feline retroviral infections diagnosed at the Ontario Veterinary College (1999-2012)

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Purpose:
It is speculated that the seroprevalence of feline immunodeficiency virus (FIV) and feline leukemia virus (FeLV) infection are evolving differently over time. The objectives of this study were to assess a) the temporal trends in the seroprevalence of these infections and b) the effect of vaccine introduction on FIV seroprevalence.

Methods:
The monthly seroprevalence of FIV and FeLV based on positive tests performed from 1999-2012 at the Ontario Veterinary College (OVC) were modeled as a function of trend (linear and quadratic functions), seasonality (first and second degree harmonics) and known risk factors (age, sex and neuter status) using Poisson regression and generalised linear autoregressive moving average (GLARMA) time series regression ($\alpha = 0.05$). To assess the effect of the vaccine introduction to control FIV, an intervention term (dichotomous variable) was added to indicate periods without (1999-2002) and with vaccine availability (2003-2012).

Results:
The overall seroprevalence for the time period was 4.5% and 3.3% for FIV and FeLV, respectively. The monthly seroprevalence ranged from 0-44.0% and 0-33.3% for FIV and FeLV, respectively. Poisson and GLARMA regression models provided no evidence for a secular trend, seasonal effect or a statistically significant FIV vaccine intervention effect. However, the proportion of males among the tested cats was a significant predictor for FIV seropositivity (RR=1.01 (95% CI: 1.01, 1.03), P= 0.02).

Conclusions:
There was no evidence to indicate that FIV and FeLV seroprevalence are declining among population served by OVC. Further, FIV vaccine introduction did not have an effect on seroprevalence of FIV.

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Antimicrobial resistance in Escherichia coli isolated from equine clinical diagnostic specimens

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Antimicrobial use is an important driver for selection of antimicrobial resistance. To date, antimicrobial resistance is an emerging and increasingly encountered phenomenon for many clinically relevant bacteria. Antimicrobial resistance has been extensively studied in humans or livestock animals. In contrast, much fewer studies addressed bacterial resistance to antimicrobials in companion animals, particularly horses. Resistance to a wide group of antimicrobial agents, including aminoglycosides, cephalosporins, fluoroquinolones, potentiated sulphonamides, and tetracyclines that equine practitioners commonly use were described in equine Escherichia coli. However, equine research has mainly focused on

methicillin-resistant Staphylococcus aureus and, therefore, very little is known about clinically significant multidrug-resistant (MDR) E. coli. The objective of the current study is to examine the occurrence of antimicrobial resistance in E. coli isolated from equine clinical diagnostic specimens submitted to the Texas A&M Veterinary Medical Teaching Hospital (VMTH) between January 1, 2009 and December 31, 2014. A total of 202 equine E. coli isolates were identified and tested for antimicrobial susceptibility using the commercially available system, TREK Sensititre (TREK Diagnostics, Cleveland, OH) and respective Clinical and Laboratory Standards Institute (CLSI) guidelines. The results demonstrate that 193 of 202 isolates were MDR E. coli. Intrinsic resistance of E. coli to rifampin was confirmed in 98% of MDR E. coli. Resistance to penicillins varied from 33% to 100%. All MDR isolates were resistant to penicillin and oxacillin, whereas only 33% and 42% of MDR E. coli were resistant to ticarcillin and ampicillin, respectively. Resistance to aminoglycosides, amikacin and gentamicin was, respectively, observed in 2 and 31% in equine MDR isolates. Importantly, no isolates were resistant to imipenem or meropenem. Finally, out of the 193 equine MDR isolates, 12 extended-spectrum beta-lactamases-producing E. coli (5.9%) were phenotypically identified.

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Utility of electronic medical record data for healthcare-associated infection detection with fever sequella

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Healthcare-associated infections (HCAI) are a major concern for infection control programs. HCAI surveillance is often complicated by variations in subjective interpretations of the patient's clinical status. Fever is a common sequella of HCAI, an objective indicator of infectious processes, and is consistently recorded in the electronic medical record (EMR). Patients that develop a fever during their stay in the hospital are more likely to have acquired HCAI. This study reports the usability of patient rectal temperatures in HCAI surveillance.

Data for rectal temperatures (> 102.5F) were extracted from the EMR for a 30 month period, during which 94,673 canine and feline visits were made to the hospital. The median length of stay for both species was 0 days. On average, there was 1 temperature recorded per day per patient. 50,926 (53.79%) visits had temperatures entered into the EMR. 92.87% of visits without recorded temperatures lasted one day or less and were excluded from the analysis. 1,122 (2.65%) canine visits and 216 (2.51%) feline visits exhibited temperature patterns suspicious for HCAI.

Examination of the dataset shows that not all data is captured by the EMR. More critical patients often receive multiple temperature checks throughout the day. However, only one temperature is entered into the EMR per day for each patient. Further, a large number of visits do not have temperatures recorded. While a large proportion of these visits last one day or less, best practices would encourage a physical exam and entry of findings into the EMR, regardless of length of stay.

In spite of missing data, patient temperatures may still be useful in detecting HCAI. Rectal temperatures are one diagnostic component used to assess patient health. While limited to one recording per day, temperatures are consistently recorded for visits of length greater than 1 day. This population of visits is at a higher risk of developing HCAI. Records of all temperatures taken during patient visits would likely improve the detection of HCAI with fever sequella.

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GRADE approach as a tool for making evidence-based decisions in veterinary medicine

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Evidence-based practice and methods for weighing available evidence in decision-making have been increasingly emphasized in veterinary medicine and agricultural practice. Methods, such as systematic review, meta-analysis, qualitative and quantitative benefit risk-analysis, are being applied to animal health questions. One of the most critical aspects of such methods is evaluating and summarizing the quality of available information. One tool that has been widely adopted, in Cochrane Reviews and in development of human healthcare guidelines, is the Grading of Recommendations Assessment, Development, and Evaluation (GRADE) approach. The software tools with GRADE offer a transparent and systematic way to evaluate and present potential biases in evidence from published literature. The GRADE approach also has a second explicitly separate step that aides in documenting the basis for recommendations based on that evidence. Two examples will be presented illustrating how GRADE can be adapted to enhance systematic reviews in animal health. Use of the GRADE approach can increase transparency of judgments made when weighing available evidence and also simplify the presentation of results.

Companion Animal Epidemiology

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Frequency, benefits and risks surrounding animals in Ohio nursing home facilities

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Animals play an increasingly important role in nursing homes providing health benefits, but may also be a source of infections and injuries for animals and patients. To-date little is known of the involvement of animals in nursing homes, practices in place to protect pet and resident health, or specific health benefits and risks. The objective of this study was to determine the frequency and avenues in which animals are utilized in the nursing home environment, practices to safeguard health, and benefits and risks of resident-animal interactions.

An anonymous online questionnaire was developed and delivered to a convenience sample of nursing home administrators in Ohio, USA from November 2014 through March 2015.

Responses from 97 facilities (approximately 5% of all registered facilities in OH) were received and adequately completed to allow for analysis. Almost all respondents (96; 99%) allowed animals in their facilities (live-in, visiting, or both), with dogs being the most frequent animal involved with facility activities (99%), followed by cats (89%), birds (74%), fish (57%), and rodents (28%). High-risk species such as reptiles/amphibians and "petting zoo farm animals (e.g., pigs, goats, chickens)" were often permitted in facilities (27% and 12%, respectively). Few facilities had restrictions targeting particular species (n=9) or age (n=1) of animals. Animals were reported to visit most (71%) facilities weekly or more often. One respondent (1%) reported an animal (cat) was mistreated by a resident or staff in the preceding 12 months. No known or suspected animal-

associated infections were reported for the preceding 12 months. Perceived benefits of animals in facilities was high (e.g., residents reported to be happier after interacting with an animal, animals useful for calming agitated residents).
These results highlight the frequent use and perceived benefits of involving animals in nursing homes, but also common gaps in established policies and best practices important for reducing resident and animal health risks. Findings can assist in the development of directed guidance documents for nursing homes to better facilitate safe interactions between residents and animals.

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Epidemiologic aspects of fecal *Salmonella* and *Campylobacter* shedding among dogs at seven animal shelters across Texas

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Approximately 70 million pet dogs live in 37% of households in the United States. Companion animals, including dogs, may serve as reservoirs for a variety of zoonotic pathogens. Salmonellosis and campylobacteriosis are among the leading foodborne illnesses in the U.S., but direct contact is also an important potential transmission route. Our objective was to assess the prevalence and diversity of *Salmonella* and *Campylobacter* shedding among shelter dogs across Texas. Using a repeated cross-sectional study design, we collected fecal samples from dogs in seven Texas animal shelters between May, 2013 and December, 2014. All 554 samples were processed for *Salmonella*, and samples from the final round of sampling (n=185) were also processed for *Campylobacter*. Detection and isolation methods included standard bacteriologic techniques and real time (RT) and traditional polymerase chain reaction (PCR) assays. *Salmonella* isolates were further characterized using molecular serotyping and antimicrobial susceptibility testing. The prevalence of fecal *Salmonella* among sampled dogs was 4.9% (27/554), and within-shelter prevalence ranged from 1.9% to 8.3%. The most common serovars were *S.* Newport and *S.* Javiana, which are among the top five serovars isolated from humans in the U.S. Antimicrobial resistance was uncommon among *Salmonella* isolates. The prevalence of *Campylobacter* using a culture-independent method (i.e., RT-PCR) was higher (75.7%; 140/185) than the prevalence using culture-dependent methods (45.4%; 84/185). Only 7.1% (10/140) were positive for *Campylobacter jejuni*, and none of the samples were positive for *Campylobacter coli*. Our results suggest that the prevalence of fecal *Salmonella* shedding among shelter dogs in Texas is comparable to that among pet dogs nationwide. *Campylobacter* prevalence was relatively high, and few canine studies in the U.S. are available for comparison. In addition, dogs are capable of shedding *Salmonella* serovars and *Campylobacter* species that are important for public health.

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An observational study of the prevalence of heartworm disease in Mississippi shelter dogs and test efficacy

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Purpose:
Heartworm disease is a progressively fatal disease in dogs. Testing in the clinical setting is considered a standard of care. Many shelters across MIssissippi lack financial means to test dogs in their care. This study was designed to determine the prevalence of the disease in MS shelter dogs, compare agreement between six heartworm detection tests, and identify risk factors for heartworm positive results.
Methods:
Fifty-seven dogs were enrolled in the study from three shelters in Mississippi. Dogs were required to be >6 months of age, apparently healthy, with no history of heartworm preventive, heartworm treatment or microfilariacide administration. Six commonly used heartworm tests were utilized to detect microfilaria or heartworm antigen. Tests included a direct blood smear, Modified Knott's test, commercially available heartworm antigen test and antigen batch test. The antigen test and batch test were also performed on heat-treated sera. The kappa statistic determined test agreement and performance compared to a commercial antigen detection ELISA. Multivariable logistic regression was used to test for associations between dog characteristics and positive test results.
Results:
Antigen was detected in 23 of the 57 dogs (40%; 95% CI 29%, 53%). All commercial tests had high agreement with a commercial ELISA (sensitivities >90%, specificities=100%, and kappa >0.90). Sensitivity was 60.87 and 80% and specificity was 97.06 and 57.14% for blood smear and Modified Knott's test respectively with kappas of 0.6138 and 0.3514. Dogs >5 years had greater odds of testing positive (odds ratio= 8.8, p=0.04). Each additional pound in body weight was also significantly associated with a positive heartworm test (odds ratio=1.041, p=0.03).
Conclusions:
The prevalence of heartworm disease in this population was higher than reports from reference laboratories for MS. Older, larger dogs were at increased odds of heartworm disease likely due to increased time of exposure and body surface. Heartworms appear to be endemic to shelter dogs. The level of agreement between the heart worm antigen tests was high, while the level of agreement between the antigen and the microfilaria tests were low.

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Progression of surgical efficiency, incision length and complication rate in senior veterinary students enrolled in a 2 week spay/neuter surgical elective

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Purpose:
Veterinary schools continually seek new ways to provide students with opportunities for education and growth, as well as ways to track the progress of their students. The purpose of this study was to evaluate 4th year veterinary student progression in surgical efficiency, incision length

and complication rate during a series of routine canine spays performed over a 2 week elective.
Methods:
Data from surgical records were collected from a 2 week senior student elective surgical rotation. Data included the signalment of the animal, the name of the student (surgeon), the surgical start time, finish time, surgical incision length and any complications encountered during surgery. The association between number of surgeries conducted by a student and surgical time or incision length was tested using linear regression in a generalized linear mixed model with a correlation structure defining clustering by student. The association between number of surgeries conducted by a student and the occurrence of serious hemorrhage or a dropped ovarian pedicle was tested using logistic regression in a generalized linear mixed model with a correlation structure defining clustering by student. A p-value of ≤ 0.05 was considered significant.
Results:
Records were evaluated from 1137 canine ovariohysterectomy surgeries conducted by 90 student surgeons. The mean number of canine spays performed by each student was 9.8, (range of 1 to 46). Surgical time was recorded on 1132 records. The mean surgical time was 43.9 minutes (range of 4 to 153). A decrease of 0.61 minutes was observed for each consecutive surgery (p= <.0001). The incision length was recorded in 1068 of the records. The mean incision length was 2.8 cm (range of 1 -11).Incision length decreased by 0.2 mm (p=0.0014) with each consecutive surgery. No significant difference was found in the occurrence of hemorrhage or dropped pedicles.
Conclusions:
Students showed significant decrease in surgical time and incision length with each surgery. Based on these observations, this elective has a positive impact on the student's surgical skills.

Companion Animal Epidemiology

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Peri-operative morbidity and risk factors associated with routine sterilization surgeries performed on a mobile surgical service

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Sterilization surgeries are essential tools for controlling pet overpopulation. Partnerships existing between teaching institutions and humane societies provide surgical experience to veterinary students and surgical services to shelters. The purpose of this observational study was to determine the peri-operative morbidity rate for animals receiving sterilization services through a mobile surgical teaching service. Four humane societies were enrolled as a representative sample of the overall group of 20+ shelters visited by the program. Surgical records kept included signalment and health status of the animal, surgical time, incision length, and surgical and anesthetic complications. Seventy-two hours and one week post-operation all animals remaining at the shelter sites were evaluated for pain and post-operative complications. Over the course of four months 472 animals were included in this study. A 4.45% (95% Confidence interval(CI): 2.93, 6.71) inter-operative complication rate occurred and included bleeding pedicles, uterine bodies, spermatic cords, other hemorrhage, damage to the spleen, and incisional complications. At 72 hours 235 animals were available for follow-up and a 12.77% (95% CI: 9.09, 17.64) post-operative complication rate was noted with the majority being seroma formation. At one week post-operative the complication rate decreased to 10.58% (95% CI: 6.96, 15.78) with 189 animals available for examination. The total post-operative complication rate for the animals from this study population available over the follow-up period was 14.11% (95% CI: 10.33, 18.99).The peri-operative morbidity rates reported by this study fall within the ranges of previous reports for surgical teaching programs. These results demonstrate that mobile sterilization services are a viable teaching and surgical model that can be utilized to reduce pet overpopulation and teach veterinary students valuable soft tissue surgical skills.

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Prevalence and characterization of *Salmonella* isolated from feral pigs throughout Texas

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Feral pigs are one of the most abundant free-roaming ungulates in the United States, yet their role in the ecology and transmission of foodborne pathogens is poorly understood. Our objectives were to estimate the prevalence of *Salmonella* shedding among feral pigs throughout Texas, to identify risk factors for infection, and to characterize the isolates. Fecal samples were collected from feral pigs in Texas from June, 2013 through May, 2015. Standard bacteriologic culture methods were used to isolate *Salmonella* from samples, and isolates were characterized via serotyping and antimicrobial susceptibility testing. The prevalence of fecal *Salmonella* shedding among sampled pigs was 43.9% (194/442), with positive pigs originating from 50 counties. Pigs sampled during fall and summer were significantly more likely to be shedding *Salmonella* than pigs sampled during winter. High serovar diversity was evident among the isolates, and many of the detected serovars are leading causes of human salmonellosis. The most common serovars were Montevideo (10.0%), Newport (9.1%), and Give (8.2%). Resistance to antimicrobial agents was rare. High prevalence of fecal *Salmonella* shedding among feral pigs throughout Texas suggests that this invasive wildlife species is an emerging threat to food safety in the United States. Entry of *Salmonella* into the food production chain can occur through fecal contamination of crops and irrigation water, pathogen transmission to livestock, and contamination of the feral pig meat itself during harvest. Transmission via direct contact is an additional risk among hunters, wildlife biologists, and others who handle feral pigs.

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Dissemination of carbapenem-resistant Enterobacteriaceae from a municipal wastewater treatment plant

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Carbapenem resistant Enterobacteriaceae (CRE) are classified as an urgent public health threat by the US CDC. Bacterial resistance to carbapenems is frequently mediated by carbapenemase genes located on highly mobile plasmids. Numerous genes, including the blaKPC and blaNDM-1 are known to encode bacteria the ability to produce cabapenemase. While both are present in the US, blaKPC has emerged and

disseminated primarily in the US, while blaNDM-1 has primarily disseminated in SE Asia. Because we have previously recovered enteric bacteria producing carbapenemase from wastewater treatment plant influent, and because wastewater is not sterilized during treatment, we hypothesized that enteric bacteria harboring plasmid-borne carbapenemase genes were discharged into the environment in effluent. During the summer of 2015, we collected samples weekly at the Jackson Pike Wastewater Treatment Plant in Columbus Ohio. Each week, one liter samples were collected pre-effluent, effluent, post-effluent, as well as upstream and downstream of discharge. Samples were filtered and enriched in nutrient broth with meropenem, incubated overnight then inoculated to MacConkey agar supplemented with meropenem to identify isolates expressing the carbapenemase phenotype. Carbapenemase-producing coliform bacteria we recovered from each sampling site, with prevalence rates ranging from 20% to 100%. The most common genotype was blaKPC although other genotypes were recovered. Our results indicate that carbapenemase-producing enteric bacteria are commonly disseminated into the environment from wastewater treatment plants. Humans, wildlife, companion animals, and livestock downstream from the discharge may be exposed and become colonized by these CREs in the environment, posing a risk to public and animal health.

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Effect of copper, zinc, and essential oil supplementation on antimicrobial resistance of fecal Escherichia coli in nursery piglets

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Copper, zinc, and essential oils are alternatives to antibiotics (ATA) that have been suggested to improve growth performance. The effects of these ATA on the resistance features of fecal Escherichia coli in nursery piglets have not been fully explored. Therefore, we compared the effects of in-feed copper, zinc, and oregano oil (alone, or in combinations) with those of in-feed low or high doses of chlortetracycline on antimicrobial resistance of fecal E. coli. Study consisted of 350 weaned piglets 21 days of age were randomly assigned to 70 pens (5 pigs per pen). On day 5, pens were randomly allotted to one of 10 treatment groups in a 2×2×2 (+2) factorial design with main effects of Cu (0 vs. 125 ppm Cu), Zn (0 vs. 3,000 ppm Zn from d 5 to 12 and 2,000 ppm Zn from d 12 to 33), and oregano oil (0 vs. 0.1%). Two additional treatment groups were fed sub-therapeutic or therapeutic levels of chlortetracycline (CTC; 55 or 441 mg/kg of feed). Fresh fecal samples were collected weekly over 42 days by gentle rectal massage from three pigs in each pen. Isolation and enumeration of E. coli was done by plating fecal samples from days 0 and 28, on MacConkey agar (MAC), MAC+ Tetracycline (16 µg/ml) , MAC+Ceftriaxone (4 µg/ml), and MAC+Cu (1 to 8 ppm) plates. A triplex PCR was done on E. coli isolates to detect tetA, tetB and blacmy-2 genes. Minimum inhibitory concentrations were determined by sensititer procedure. Whole genome sequencing was performed on a subset of isolates to compare genotypic data with phenotypic resistance. The data were analyzed using generalized linear mixed models (STATA MP v. 12.1). Copper, zinc, and oregano oil did not show any effects on expanding antibiotic resistance; in fact, copper fed alone had a sparing effect on multi-drug resistance and ceftiofur resistance. A majority of the isolates were resistant to tetracycline (99%) while 5.7% of the isolates were positive for qnrB gene and resistant to ciprofloxacin.

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Antimicrobial susceptibility of enteric Gram-negative facultative anaerobe bacilli in aerobic versus anaerobic conditions

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Purpose: Antimicrobial drug use in farm animals results in antimicrobial exposure of the host's enteric bacteria, some of which are potential foodborne pathogens. Assessing how this exposure impacts the enteric bacteria necessitates development of pharmacodynamic models of the drug action against the bacteria in the anaerobic conditions of intestine. The models require measurements of bacterial antimicrobial susceptibility, such as the minimum inhibitory concentrations (MIC) of the drugs. Currently, the measurements obtained in aerobic conditions in vitro are utilized. However, the Gram-negative bacilli among foodborne pathogens, Escherichia coli and Salmonella, are facultative anaerobes which experience physiological changes in anaerobic conditions. The objective of this study was to investigate differences in antimicrobial susceptibility under aerobic and anaerobic conditions of generic E. coli and Salmonella isolates from cattle feces. We focused on bactericidal antimicrobials and included all such drug classes used in cattle: cephalosporins, older β-lactams (aminopenicillins), aminoglycosides, and fluoroquinolones.

Methods: The susceptibility of each bacterial isolate to ceftriaxone, ampicillin, kanamycin, gentamycin, and enrofloxacin was measured using E-test method under aerobic conditions and anaerobic conditions following a 24-hour period of adaptation of the bacterial culture. A total of over 80 isolates of E. coli and Salmonella were tested.

Results: The susceptibility of E. coli and Salmonella isolates to aminopenicillins, aminoglycosides, and fluoroquinolones in anaerobic conditions differed from that in aerobic conditions. This was observed for the isolates susceptible (based on the clinical breakpoint interpretation) to the studied antimicrobials when tested in aerobic conditions.

Conclusion: The results demonstrated that for modeling and assessing the impact of antimicrobial use in cattle on their enteric bacteria that are Gram-negative facultative anaerobe bacilli, the measurements of bacterial susceptibility to the antimicrobials reaching the intestine may need to be obtained in anaerobic conditions.

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Comparing the resistome of poultry, swine, cattle and salmon production and nearby human waste water treatment plants

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Purpose:

Metagenomics holds immense potential for understanding how antimicrobial resistance (AMR) genes move within and between livestock

production systems and human habitats. The ability to interrogate the entire resistance potential of a given sample also enables comprehensive investigation of how different livestock production processes - including different antimicrobial use protocols - impact the risk of AMR transmission to humans.
Antimicrobial use patterns differ widely between poultry, swine, cattle and salmon production in North America. We hypothesized that the resistome of poultry, swine, cattle and salmon feces would differ and that these differences could inform design of waste management processes tailored to each production system. Furthermore, we hypothesized that the resistome of biosolids from human wastewater treatment plants (WWTPs) located near these facilities would differ from the feces of each commodity.
Methods:
To test these hypotheses, we collected composite fecal samples from large commercial poultry and swine barns, farmed salmon sea cages, and feedlot cattle pens; in addition, we collected treated biosolids from nearby human WWTPs. Total DNA from 5 samples from each site (total N = 25) was extracted and shotgun sequenced on the Illumina HiSeq. Reads were compared to a database of AMR gene sequences to characterize the resistome in each sample. Resistome composition was compared using non-metric multi-dimensional scaling ordination. Abundance of different AMR mechanisms and classes were compared between samples using zero-inflated Gaussian mixture models.
Results:
Shotgun sequencing produced ~5.5 billion reads across all 25 samples. Fewer than 5% of reads were removed due to low quality, and &gt 200 AMR genes across all samples. Resistome composition differed by commodity species, and the resistome of human WWTP samples clustered separately from all livestock samples.
Conclusions:
These results demonstrate that the fecal resistomes of chicken, swine, beef cattle and salmon differ significantly, suggesting that manure management systems may need to be tailored for each commodity system.

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Use of shotgun metagenomic to evaluate the microbiome in cattle feces following tulathromycin metaphylaxis

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Shotgun metagenomics, facilitated by next-generation sequencing, represents a novel approach to investigate microbial communities. The goal of this study was to use a metagenomic approach to understand the impact of metaphylactic tulathromycin exposure on the microbiome of cattle in the early feeding period. Two pens of cattle in a Texas feedlot were selected for this study. One pen was chosen to receive 800 mg of tulathromycin while the other was chosen for the control. Individual fecal samples from the rectum were collected at arrival processing and 11 days into the feeding period. Fecal samples from treated (n=30) and control (n=30) animals from both sampling times were subjected to DNA extraction for metagenomic sequencing. After sequencing, low quality sequences and bovine DNA were removed using Trimmomatic and Burrows-Wheeler Aligner softwares, respectively. Then, a taxonomic sequence classifier (Kraken) was used to assign taxonomic labels to non-bovine DNA sequences. Kraken aligns sequenced reads to the Reference Sequence database (National Center for Biotechnology Information, NCBI) and determines the bacterial composition of samples based on nucleotides matches. Next, the number of reads that map to different NCBI taxonomic labels were normalized to account for differences in sequencing depth across samples and get an estimate of the relative abundance of each taxonomy group. A non-metric multidimensional scaling ordination was used to determine whether the overall microbiome differed between groups of cattle. In order to identify specific taxonomic labels that were significantly different between groups, multivariate models were built using zero-inflated Gaussian mixture distributions. The proposed pipeline successfully characterized the microbiome in the feces of cattle with or without exposure to metaphylactic tulathromycin. However, more comprehensive genome databases are required to strengthen the classification of metagenomic reads. Scientists and industry partners need to join efforts to understand the use of metagenomics in food production environments and to develop a better understanding of the biological relevance of the metagenomic results.

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Characterizing variation in the microbial resistome between natural and conventional beef operations

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Routine antibiotic use has drawn criticism and led to the creation of alternative practices in food animal production, such as the “natural” label, which establishes guidelines for decreased hormone and antibiotic use. It is thought that less antimicrobial use will lead to less selective pressure for Antimicrobial Resistance (AMR) and result in a more secure food supply. However, direct characterizations of microbial populations in feedlots are rare, and more evidence is needed to support this claim. This study aims to characterize the microbial communities and resistomes in natural and conventional beef feedlots using metagenomics.
Composite fecal (N=12) and wastewater samples (N=13) were taken from four feedlots in Western Canada. One feedlot contained both conventional and natural cattle. Engineered wetland (N=1) and human wastewater treatment plant (N=6) samples were taken from the surrounding area. Total DNA was extracted and sequenced on the Illumina HiSeq platform. Low quality bases were removed using Trimmomatic. Host DNA was removed by filtering reads that aligned against the Bos taurus genome using BWA. The remaining reads were profiled by alignment against our AMR gene database using BWA. AMR gene composition was validated using Hidden Markov Models trained on our AMR database and applied to the raw sequencing reads. Microbiomes were profiled using Kraken and MetaPhlAn. The coverage of functional pathways was determined using HUMAnN2.

Microbial profiles and resistomes varied significantly between the fecal composites from the natural and conventional feedlots. Conventional fecal samples had a higher relative proportion of AMR mechanisms and lower relative microbial diversity than did the natural samples. Other sample types also varied between natural and conventional but less so than the fecal samples. Microbial community profiles and pathways varied significantly by sampling location and were corrected for using generalized linear modeling.
Conventional methods and antibiotic use could contribute to the significant variation in the microbial profiles and resistomes associated with different types of production practices.

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Repeated oral immunization with Shiga toxin negative Escherichia coli O157:H7 transiently reduces carriage of wild-type EHEC O157 by cattle following oral challenge

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Pre-harvest intervention on cattle farm in reducing the shedding of enterohemorrhagic Escherichia coli O157:H7 (EHEC O157) is an important step in reducing the incidence of EHEC O157 in human. Vaccination of cattle reduces EHEC O157 fecal shedding density and duration. However, most of these vaccinations use multi-dose injectable vaccines making it impractical and hard to be adopted for regular use on cattle farms. Live oral vaccines, administered in feed permit repeated vaccination without animal restraint. The objective of this study was to determine the effects of repeated oral exposure to live Stx-, LEE+ EHEC O157 on colonization by wild-type (WT) EHEC O157. Cattle (n=20) in two equal groups were orally immunized twice weekly for six weeks with 3x109 CFU containing three stx-, LEE+ EHEC O157 strains (vaccine group) or three stx-, LEE-, non-O157 E. coli (sham-vaccine group). Three weeks following the final oral administration, animals in both groups were challenged with a cocktail of four, stx+, LEE+ WT-EHEC O157 strains. The two groups of cattle were compared for weekly recto-anal junction mucosa (RAJ) colonization density and duration with the WT-EHEC O157 strain for four weeks following challenge with WT EHEC O157. Serum antibodies against TTSP, Tir and EspA on day 0 (pre-immunization), day 61 (post-immunization, pre-challenge) and day 89 (post-challenge) were also determined by ELISA. The results demonstrate that the RAJ EHEC O157 colonization was significantly lower in vaccine group cattle as compared to the sham-vaccine group cattle ($P < 0.05$), mostly during the first week after challenge. Following immunization (vaccine group) or challenge (control group), all cattle seroconverted to have antibody against TTSP, Tir and EspA. Antibody titers against Tir and TTSP following immunization (day 61) were associated with decreased fecal numbers of WT-EHEC O157. These results suggest oral vaccination against EHEC O157 with live bacteria expressing LEE, with an increased the efficacy and duration, has potential to be used as an inexpensive bovine immunization that will not require animal handling and restraint.

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Experiential learning: The Farm to Table Study Program as a case study

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Experiential learning, or learning through structured experience involving reflection and analysis, can lead to multiple positive learning outcomes, including deeper understanding of content material as well as development of group leadership skills. The Farm to Table Study Program, offered annually for the past seven years in an exporting Latin American country through collaboration between the University of Minnesota, Ohio State University, and a local university, provides participants the opportunity to explore firsthand animal food production systems from farm-to-table. Site visits along the food supply chain are focused on production, international trade standards, and emerging issues in food safety and animal and public health. Direct discussions with key government and private sector leaders and interactive multi-sectorial and cross-culture group activities are designed to integrate knowledge and skills. This program is an example of the type of systems-based experiential learning needed by the next generation of public health and food systems professionals.

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Risk profile and quantitative exposure assessment of hepatitis E virus from pigs or pork in Canada

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Purpose:
The role and importance of pigs and pork as sources of zoonotic hepatitis E virus (HEV) has been debated in Canada and abroad for over 20 years. Therefore, we compiled data to populate a risk profile (RP) for HEV in pigs or pork in Canada.
Methods:
We organized the risk profile using the headings prescribed for a foodborne microbial risk assessment. We used research synthesis methods and inputs wherever possible in populating the fields of this RP. A scoping review of potential public health risks of HEV, and two Canadian field surveys sampling finisher pigs, and retail pork chops and pork livers provided inputs to inform this RP. We calculated summary estimates of prevalence using the Comprehensive Meta-analysis 3 software, employing the method of moments. We fitted exposure models using @Risk software with a 10,000 iteration burn-in and a 50,000 iteration Monte Carlo simulation.
Results:
Overall, we found the incidence of sporadic locally acquired Hepatitis E in Canada, compiled from peer reviewed literature (n = 2 cases) or from diagnosis at the National Microbiology Laboratory (n = 12) to be low relative to other non-endemic countries. In contrast, we found the

prevalence of detection of HEV RNA in pigs, and retail pork livers, to be comparable to that reported in the USA and Europe. Quantitative estimates of risk of foodborne HEV from exposure to pigs/pork were not possible to estimate, owing to data gaps including dose-response data. However, we drafted risk categories (high/medium/low) for exposure to both pigs and pork, based on likelihood of exposure, and presence or absence of known risk factors.
Conclusions:
We hypothesize that the proportion of the Canadian population at high risk of Hepatitis E via either exposure, is relatively small.

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Mark Gearhart Memorial Award, Salon A/B/C/D Performance and carcass characteristics of commercial feedlot cattle from a study of vaccine and direct-fed microbial effects on Escherichia coli O157:H7 fecal shedding
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The objective of this study was to quantify cattle performance and carcass characteristics associated with administration of a siderophore receptor and porin proteins-based vaccine (VAC) and a direct-fed microbial (DFM), which were originally evaluated for their impact on Escherichia coli O157:H7 fecal shedding in a commercial feedlot population. Cattle (n = 17,148) were randomly allocated into 40 pens grouped by allocation dates into 10 complete blocks; pens within block were randomly allocated to control, VAC, DFM, or VAC + DFM treatment groups in a 2 × 2 factorial design. The DFM (Bovamine) was fed daily at the labeled dose of 106 cfu/animal of Lactobacillus acidophilus for the duration of the intervention period (mean = 86.6 d). The VAC cattle were vaccinated on Days 0 and 21 whereas unvaccinated cattle were not given a placebo or rehandled on Day 21. Data were analyzed using general and generalized linear mixed models that accounted for the study design. Main effects of DFM and VAC are reported as there were no significant treatment interactions for any of the outcomes evaluated. Vaccinated cattle had lower total weight gain ($P < 0.01$), ADG ($P = 0.03$), and cumulative DMI during the intervention period ($P < 0.01$) compared with unvaccinated cattle, whereas the DFM increased total weight gain ($P = 0.03$) and G:F ($P = 0.05$) during the intervention period. Daily DMI was decreased ($P < 0.01$) in vaccinated pens compared with unvaccinated pens during a 5-d period immediately following revaccination. After the intervention period was completed, cattle were sorted following the standard operating procedure for the feedlot and all cattle were fed the DFM from that point until harvest. Each steer was individually identified through harvest. At harvest, vaccinated cattle had more total days on feed ($P < 0.01$) with a larger HCW ($P = 0.01$) than nonvaccinated cattle, whereas cattle not fed the DFM during the intervention period had a significantly larger HCW ($P < 0.01$) than those fed the DFM during the intervention period. We conclude that the use of these DFM and vaccine products have differential and independent effects on cattle performance and carcass characteristics in a commercial feedlot setting.

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Trends in antimicrobial resistance patterns of common Salmonella serotypes isolated from bovine samples in Wisconsin from 2006-2015
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Purpose: In the United States, Salmonella enterica subspecies enterica causes an estimated 1.2 million cases of illness, thousands of hospitalizations and approximately 400 human deaths, annually. Contributing to the morbidity and mortality is increased incidence of multi-drug resistant isolates. Because the majority of human infections are zoonotic, increasing attention is being paid to trends in antimicrobial resistance of Salmonella isolates from food animals. This study aimed to describe the antimicrobial resistance patterns for various Salmonella serotypes isolated from bovine samples submitted to the Wisconsin Veterinary Diagnostic Laboratory (WVDL).
Methods: Salmonella serotyping and antimicrobial susceptibility data was obtained from the laboratory information management system at the WVDL. A total of 4,448 accessions were included in this study. Data from accessions were limited to bovine samples submitted to the WVDL from Wisconsin between January 2006 and June 2015 and had both a definitive serotype and complete results for antimicrobial susceptibility testing. Data analysis was conducted using statistical software (STATA).
Results: Preliminary results show that Salmonella serotype Dublin is the most prevalent serotype identified amongst cattle in Wisconsin in the time period investigated. Salmonella serotypes Cerro, Kentucky, Montevideo and Newport were also among the 5 most common serotypes. Antimicrobial resistance in Salmonella Dublin, as quantified by TREK Sensititre®, varies by antibiotic class. Resistance to penicillins and tetracyclines, although high, is decreasing; while resistance among fluoroquinolones remains low, with little to no fluctuation. Of particular zoonotic importance in this group, Salmonella Newport shows increasing resistance among penicillins, tetracyclines and certain aminoglycosides.
Conclusions: These data show that antimicrobial sensitivity for some of the most common bovine Salmonella isolates has changed in Wisconsin in the past 10 years. This is important for Salmonella disease ecology in Wisconsin, which is critical for human and veterinary medical practice and preventative medicine.

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Temporal and geo-spatial characterization of Salmonella enterica serotypes isolated in Wisconsin from 2006 to 2015
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Purpose: Cattle can be both in-apparent carriers and clinically susceptible to Salmonellosis. In both cases, the pathogen is shed by cattle propagating infection on the farm and creating a significant zoonotic risk from waste runoff into water and contamination of food. The objective of this study was to determine the frequency and concurrent location of *Salmonella enterica* subspecies *enterica* serotypes isolated from bovine

diagnostic samples in Wisconsin. We also aimed to link other geographic variables to isolate location and frequency in Wisconsin with geographic information system (GIS) technology.
Methods: *Salmonella* serotypes and zip codes were collected retrospectively from accessions of diagnostic samples using the laboratory information management system at the Wisconsin Veterinary Diagnostic Laboratory. Accessions were limited to bovine fecal and tissue samples that yielded a typeable *Salmonella* serotype either by culture or polymerase chain reaction (PCR) and those originating in Wisconsin from 2006 to July 2015. An accession was counted as single incident even if it included positive samples from multiple animals. For each incident, the zip code was used to plot the geographic location of the isolated *Salmonella* serotype in ArcGIS, and yearly trending information on the incidence and location of the major serotypes was statistically analyzed.
Results: 5,122 accessions were included in this study that yielded typeable *Salmonella enterica* serotypes. The five most frequently isolated serotypes were Dublin, Cerro, Newport, Montevideo and Kentucky. With statistical analysis and visualization by GIS, the highest concentration was observed in Fox Valley area followed by northwestern and south-central parts. However, year-to-year variation in zip-code scale was not significantly different within these locations.
Conclusions: These data indicate that environmental and management factors could be driving the incidence and clustering of certain *Salmonella* serotypes in Wisconsin with dense populations of cattle. The data suggest that further investigation on factors and linkage with human infection data is warranted.

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Genotypic characterization of extended-spectrum cephalosporin resistant nontyphoidal Salmonella from the NAHMS Feedlot 2011 study

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In the US, nontyphoidal Salmonella are a common foodborne zoonotic gastroenteritis pathogen. Invasive Salmonella infections caused by extended-spectrum cephalosporin resistant (ESCR) phenotypes are more likely to result in treatment failure and adverse health outcomes, especially in severe pediatric Salmonella infections where the extended-spectrum β-lactams are the therapy of choice.
To examine the genetic characteristics of ESCR Salmonella which may enter the food chain, we characterized 44 ceftiofur-resistant Salmonella isolates from the National Animal Health Monitoring System (NAHMS) 2011 beef cattle feedlot heath and management project.
As part of the NAHMS Feedlot study, 5,050 individual fecal samples from 68 large (1,000+ head capacity) feedlots were cultured for Salmonella spp. The resulting 460 positive samples yielded 571 Salmonella isolates with 111 samples (24%) having multiple serotypes. The most prevalent serotypes were S. Anatum (n=103, 18%), S. Montevideo (n=98, 17%), and S. Kentucky (n=87, 15%).
Of the 571 feedlot Salmonella isolates, 44 (8%) expressed an AmpC β-lactamase phenotype, but no isolates exhibited an ESBL phenotype. The 44 phenotypic blaCMY Salmonella isolates represented 8 serotypes, most commonly S. Newport (n=14, 32%), S. Typhimurium (n=13, 30%), and S. Reading (n=5, 11%), followed by S. Dublin, S. Infantis, S. Montevideo, S. Rough O:i;v:1;7, and S. Uganda.
Carriage of the blaCMY gene was confirmed for all isolates by PCR. Additionally, all 44 isolates were PCR-positive for the presence of an Inc A/C plasmid which has been previously reported to harbor blaCMY in multiple species. Other plasmids, including Inc N, FIC and FIIA, were also detected in some isolates.
Most Salmonella infections are the result of zoonotic foodborne transmission from livestock reservoirs where extended-spectrum cephalosporins are commonly used. Our characterization of the NAHMS Feedlot Surveillance ESCR Salmonella shows that while other cephalosporin resistance mechanisms have been reported in US cattle, specific serotypes harboring blaCMY on Inc A/C plasmids may be the dominant ESCR genotype.

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Extended-spectrum cephalosporin and fluoroquinolone-resistant enterobacteriaceae in human and veterinary hospital environments

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The dissemination of Enterobacteriaceae expressing resistance to extended-spectrum cephalosporins and fluoroquinolones is of critical concern to both human and veterinary medicine. The common use of these antimicrobials provides selection pressure favoring resistant bacteria in the patient's enteric flora. In healthcare facilities, the movement of patients, personnel, and equipment provides an opportunity for Enterbacteriaceae carrying resistant genes to enter the environment. Once in the hospital environment these strains can be disseminated both within and beyond the medical facilities.
We collected environmental samples using electrostatic clothes to assess the prevalence of resistant Enterobactereriaceae on human and veterinary hospital environmental surfaces. Samples were collected with individual cloths in multiple hospital services, with the same surfaces sampled on each visit. Collected samples were enriched with nutrient broth with cefotaxime, incubated then inoculated to 5 MacConkey agars supplemented with cefoxitin, cefepime, meropenem, naladixic acid, and ciprofloxacin to select clinically important resistance phenotypes.
A total of 31 contact surfaces were sampled at each of 4 visits to 2 human hospitals (n=124). Additionally, samples were collected from 37 surfaces on 3 visits to 3 veterinary medical centers, treating both large and companion animals (n=111). Isolates expressing the AmpC phenotype were found on 14.5% of human and 55.9% of veterinary hospital surfaces. Phenotypic ESBL-producers were recovered from 1.6% of human and 33.3% of veterinary surfaces. CREs were detected from 2 surfaces in human medicine environments (1.6%). Naladixic acid resistant isolates were found on 2.4% of humans and 43.2% of veterinary surfaces. Ciprofloxacin resistant isolates were found on 0.8% of humans and 39.6% of veterinary hospital environments.
Our results indicate that antimicrobial resistant Enterobacteriaceae can contaminate surfaces in both human and veterinary medical settings with higher prevalence observed in veterinary hospitals. The recovery of CRE from human hospital environments is concerning and warrants further investigation.

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Spatial clustering of cefotaxime and ciprofloxacin resistant E. coli among dairy cattle relative to the European starling night roosts

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European starlings (Sturnus vulgaris) contaminate livestock facilities with their droppings and disperse zoonotic enteric pathogens. Antimicrobial resistant Escherichia coli (E. coli), Salmonella and Camplobacter jejuni have also been isolated from starlings. However, their role in disseminating antimicrobial resistant organisms has not been explored. In a previous study, starling night roosts were identified as potential foci for the dissemination of E. coli O157:H7 among dairy farms. Subsequently, our aim was to determine whether these night roosts could be centers for spreading antimicrobial resistant organisms among livestock operations. Bovine fecal samples were collected from 150 dairy farms visited twice (in summer and fall) between 2007 and 2009. A total of 1490 samples (10 samples/farm) were tested for ciprofloxacin and cefotaxime resistant E. coli. Using the spatial scan statistic, focal scans were conducted to determine whether clusters of resistant organisms were centered around starling night roosts. Statistically significant ($P < 0.05$) cefotaxime and ciprofloxacin resistant E. coli clusters were identified around the night roosts. This finding indicates that the risk of carriage of antimicrobial resistant organisms in cattle closest to starling night roosts was higher compared to those located on farms further from these sites. Starlings might have an important role in spreading antimicrobial resistant E. coli to livestock environments potentially threatening animal and public health.

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A survey of case-control studies in veterinary science

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The case-control study design is commonly used in veterinary sciences owing to the relatively low expense and potentially quick data collection phase. Case-control studies are most useful to clinicians when the disease or outcome of interest is rare or the exposure is expensive to measure. Decisions regarding the approach to selecting cases and controls will affect whether the study estimates the population odds, risk, or rate ratio. For example, cases may be incident or prevalent and controls can be selected at the start of the study, from the risk set, or from survivors. However authors are often unaware of the issues and the impact on the effect measure estimated by the case-control design. This lack of understanding can be an issue when systematic reviews exclude case-control studies that are actually of more similar design to cohort studies. The objective of this study was to understand the magnitude of misclassification of effect measures from case control studies and to describe how case control studies are conducted in the veterinary sciences. Using a randomly selected subset of 100 self-described case-control studies we applied two design classification systems to itemize each report and determine the study design employed. The CABI and MEDLINE databases were searched for all manuscripts labeled as case-control in select companion and livestock animals. Preliminary results suggest that confusion exists in the veterinary literature regarding design descriptions, as a sizable proportion of the sampled reports are actually cohort studies or diagnostic test evaluations. Many authors incorrectly identified the effect measure estimated by the study. Further, authors' descriptions of directionality and timing (prospective, retrospective) were often misleading or incorrect for a number of the sampled reports. The results of the assessment suggest a need for better education of veterinary researchers about observational study design and that excluding studies from reviews based on authors reports of the design may exclude relevant papers.

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Risk of a first and recurrent case of pathogen specific clinical mastitis

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The objective of this study was to determine the risk of a first and recurrent case of pathogen specific clinical mastitis (CM) in order to parameterize a pathogen specific CM economic decision model providing dairy farmers with optimal management decisions. Pathogens studied were *Escherichia coli*, *Staphylococcus aureus*, *Staph.* spp., *Streptococcus* spp., *Klebsiella* spp. and *Trueperella pyogenes*. A total of 40,864 lactations (19,835 cows) were analyzed: 17,265 were primiparous and 23,599 were multiparous lactations, from 5 large, high milk producing dairy herds in New York State. We estimated the effects of parity, calving diseases, milk yield, current season and number of CM cases in the previous lactation on the risk of a 1st case of CM. Statistical models developed include generalized linear mixed models with a log link and Poisson error distribution, zero inflated Poisson distribution models and negative binomial and logistic regression models. While logistic regression would be an obvious choice, we explored analyzing the data with the Poisson error distribution, as the unit of analysis was relatively short, therefore diminishing the distinction between risk and rate. The aforementioned effects and the occurrence of previous cases were evaluated as risk factors for 2nd and 3rd cases of pathogen-specific CM. Cows were at greater risk of pathogen-specific CM in the current lactation if they had more CM cases in the previous lactation; e.g., multiparous cows were 5.9 times [95% CI: (3.8, 9.3)] more at risk of a 1st case of *Klebsiella* spp. if in the first two weeks of lactation they had ≥ 3 cases of CM in the previous lactation, compared to cows that had no CM in the previous lactation. Multiparous cows were at greater risk of a 2nd CM case if they had suffered from a 1st case of CM that was caused by the same pathogen as the 2nd case; e.g., multipara in weeks ≥ 3 were 5.9 times [95% CI: (4.5, 7.8)] more likely to have a 2nd case of *Staph. aureus* CM if the 1st case was also *Staph. aureus*. The estimation of risks was integral to describing the biological behavior of pathogen specific CM transmission in the economic model.

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Pregnancy loss attributable to mastitis in first lactation Holstein cows

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Pregnancy loss (PL) can cause economic burden to dairy farmers due to repeat breeding, a prolonged calving-to-conception interval, or culling of dairy cows. In the USA, prevalence of PL in dairy cows has varied from 6 to 39%. Two studies have provided experimental evidence that mastitis can cause embryonic mortality and PL through the release of lipopolysaccharide (LPS) and other molecules of bacterial origin that can activate inflammatory and immune responses. Results from a systematic review of epidemiologic studies, however, show inconclusive evidence that there is a temporal relationship between mastitis and PL in dairy cows. A case-control study is underway to examine the relationship between mastitis (alone or in combination with other factors) and PL in first lactation Holstein cows.

Exposure to mastitis was measured 4 weeks before breeding, the week of breeding, 1-4, 5-6, and 7-10 weeks after breeding. A total of 992 cows that calved on one dairy in Florida between 2006 and 2013 were considered for inclusion. The study includes 104 cows (cases) with a diagnosis of PL and 667 control cows matched by time to conception. Pregnancy diagnosis in study cows was performed by ultrasound 32-33 days after breeding. Confirmation of pregnancy was conducted by the attending veterinarian using trans-rectal palpation 2 and 6 weeks after pregnancy diagnosis.

Using conditional univariable logistic regression analysis, the variables for clinical mastitis the week of breeding or 5-6 weeks after breeding (two weeks after pregnancy diagnosis), ketosis, body condition score (≤ 2.75) between breeding and pregnancy diagnosis, breeding season (summer), and breeding type (embryo transfer) had values of $p < 0.20$ and will be further examined in a multivariable analysis of PL. Preliminary results have revealed that the odds of PL were 5.8 times higher in cows with clinical mastitis during the week of breeding (95% CI = 0.93, 36.11), compared to cows without mastitis during that time period, and 3.5 times higher in cows with clinical mastitis 5-6 weeks after breeding (95% CI = 1.15, 10.7), compared to cows without mastitis.

Final results and conclusions will be presented at the conference.

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The value of pathogen information in treating clinical mastitis.

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The objective of this study was to evaluate the financial value of different methods used in identifying clinical mastitis (CM) in dairy cows by estimating the change in net returns/year with additional CM information, while accounting for the costs in obtaining more detailed information. The most basic form of identifying cows with CM is to observe outward signs (Base model). Another approach is to identify whether the pathogen causing CM is Gram positive, Gram negative or Other (Gram model). The most detailed level of information currently available for mastitis diagnostics is obtainable by sending milk samples for culture to an external laboratory, which will identify the pathogen causing CM, often within 24 hours (Culture model). Knowing the exact pathogen involved will permit treatment to be specifically targeted, resulting in less discarded milk due to potentially inappropriate antibiotic use, however, at the cost of additional waiting time for receiving results which delays treating cows and also the cost of culture. This brings into question the financial benefit of pathogen specific CM information. The economic model used was built using the Multi Level Hierarchic Markov Process software as the application program. Preliminary data demonstrate the model predicting the value of CM culture information at the pathogen specific level (Culture model) provides a greater net returns/year than other levels of information even when accounting for fluctuations in key market prices. For example, net returns/year was lowest for the Gram model, was greater for the Base model (with an added value of information (VOI) of 2.42USD) and greatest for the pathogen specific CM Culture model (added VOI of 3.59USD) compared with the Gram model when milk price was increased and decisions were made based on treatment cost. Similar results were observed with fluctuations in cow replacement cost. These results quantify the assumptions under which the pathogen specific CM Culture model provides the greatest value for optimal decision making while accounting for the cost of obtaining this additional information.

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The effect of calf gender and age of dam on the risk for calves to develop bovine respiratory disease prior to weaning

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The objective of this study was to test if calf gender, birth weight or age of dam were associated with bovine respiratory disease (BRD) of calves prior to weaning. Health records representing 9,921 calves from 28 cattle management groups within 7 Nebraska beef cattle ranches were analyzed using multilevel multivariable logistic or linear regression in generalized linear mixed models with a correlation structure defining clustering of management groups within ranch. Diagnosis of BRD was modeled using a logistic regression with explanatory variables of calf gender, birth weight, and age of the dam. The age calves were diagnosed with BRD was modeled using linear regression. Both models were built using manual forward selection with significance set at alpha ≤ 0.05. The gender of the calf was recorded for 9,705 calves: 1041 bulls, 4229 heifers, and 3409 steers. Birth weight was recorded for 4,098 calves (mean 38kg, SD 5.9kg) .The age of the dam was recorded for 9,037 calves (median 4 years; range 2 to 16 years; mean 4.4 years). Bovine respiratory disease was diagnosed in 1,031 of 9,921 calves (10.4%). Calf gender was significantly associated with BRD using 9,705 calf records. Bulls (OR = 1.23) and steers (OR = 1.19) were more likely than heifers to develop BRD prior to weaning. Birth weight and the age of dam were not significantly associated with BRD diagnosis. Age of the dam was

significantly associated with the age that 1,021 calves were diagnosed with BRD. Calves with two year old dams were diagnosed with BRD 12 days earlier than calves born to cows 3 years or older (P=0.0002). Sex and birth weight were not significantly associated with the age of BRD diagnosis. The results of this study suggest that there are sex related differences in the risk for calves to develop BRD prior to weaning. Dam age is also an important factor affecting the age of calves getting BRD. This may mean that management strategies to minimize BRD risk should consider factors of calf gender and dam age.

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An update and model assessment of a mixed treatment comparison meta-analysis of antibiotic treatment for bovine respiratory disease

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Bovine respiratory disease (BRD) is the most costly disease of beef cattle. Numerous antimicrobials are currently registered for the treatment of BRD. However, often there will not be publically available data for the treatment comparisons of interest. An MTC meta-analysis is an extension of pairwise meta-analysis that uses a network of comparisons. An MTC meta-analysis combines direct and indirect estimates of efficacy from the network of trials in order to provide comparative efficacy estimates in the absence of publically available trial data. This analysis can result in an invaluable tool for producers and veterinarians in both guiding treatment decisions as well as ranking available interventions based on relative effectiveness. In 2013, members of our group published a MTC meta-analysis to assess comparative efficacy and rank antibiotic treatments for BRD in feedlot cattle. The antibiotic, gamithromycin was only represented by one placebo comparison trial in the 2013 model. Since that time, three trials comparing gamithromycin to other antibiotics have been published. The objectives of this current project were to update the network of BRD trials and to compare indirect efficacy estimates for gamithromycin from the original 2013 model with results from subsequently reported randomized trials. The 2013 model-predicted risk ratios and credibility intervals substantially overlap with the subsequently published direct estimates of efficacy from randomized trials. This demonstrates the success of the model in accurately predicting comparative efficacy. The results of this study serve both to strengthen the clinical decision making tool for treating BRD and advance our understanding of applying complex research synthesis methods to clinical decision making in the veterinary sciences.

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An investigation of piglet iron status at weaning and subsequent post-weaning growth performance

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For many decades piglets have been given a 200 mg intramuscular (IM) injection of an iron supplementation, typically iron dextran or gleptoferron, within the first 3 days of life. However, given modern farm management improvements and advancement in genetic growth potential, piglets are being born into even larger litters and are growing even more efficiently than before. A re-assessment of this standard iron supplementation protocol is needed to determine if it still meets the needs of today's fast growing pigs. The objectives of this study were to assess the iron status of piglets pre-weaning and how this status subsequently affects nursery growth performance.

Twenty litters from 20 Ontario swine farms were sampled with one small, medium and large size piglet selected from each litter (n=1095 piglets). An individual body weight and blood sample were taken from each pig 1-2 days prior to weaning and repeated 3 weeks later when the piglets were in the nursery. Hemoglobin and other blood iron indicators were used to evaluate iron status and to examine for associations with post-weaning nursery performance (multilevel linear regression). Hemoglobin status was categorized as follows: normal Hb (>110g/L), iron deficient (>90 g/L but ≤110g/) or anemic (≤90g/L).

All farms had piglets with low hemoglobin values prior to weaning and there was a higher prevalence of anemic pigs in the nursery. Piglets that were anemic at weaning had a 0.82 kg reduction in their 3-wk post-weaning body weight (P<0.05). The large size piglets at weaning had lower hemoglobin, serum iron, hematocrit, mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration levels and higher total iron binding capacity when compared to the small size piglets (all P< 0.05). This study indicated that a single 200 mg IM injection of iron did not provide sufficient supplementation for the large, faster growing pigs and that suboptimal iron status negatively impacts nursery performance.

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A transboundary, epidemiologic simulation model for the spread and control of classical swine fever among commercial swine in the United States and Canada.

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Classical swine fever (CSF) is a highly contagious viral disease in swine that was eradicated from the United States (U.S.) and Canada in 1978 and 1963, respectively. Following eradication, significant changes in the swine industry have introduced uncertainties that complicate efforts to prepare for future outbreaks. Epidemic simulation models can be used to determine the scope and impact of a potential outbreak, as well as to evaluate control and response measures. The purpose of this study is to simulate the transboundary spread and control of CSF among commercial swine in the U.S. and Canada. Specifically, this study examines the epidemiologic impact of international trade restrictions on live swine between the U.S. and Canada. A database of U.S. and Canadian farms was created using census data. Disease parameters were developed from current literature and a within-herd disease spread model. Movement parameters for direct and indirect contacts were developed from a review of current literature, agricultural and economic reports, import data, and agricultural surveys. Movement restrictions, control zones and other disease control parameters were developed based on emergency response plans for each country and expert opinion. Disease spread is simulated with or without allowing international trade of live swine to continue following infection of an index farm in either the U.S. or Canada. Independent variables

include size of the control zone and implementation time of cross-border movement restrictions. Simulations are performed using InterSpread Plus, a spatially-explicit, stochastic modeling framework. Model outcomes include median outbreak duration, median number of infected farms and animals, the impact of cross-border animal movements on the extent of the outbreak, and the number of animal movements prevented by movement restrictions. Results from this research can provide a basis for an economic analysis of the costs and benefits of zone recognition policies for CSF between the U.S. and Canada. In addition, the epidemiological data produced from this research may be helpful in the development of emergency response policies and control measures for CSF.

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Quantification of strategies to mitigate animal-welfare concerns due to movement restrictions during a classical swine fever (CSF) outbreak

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Objective: To estimate the number of swine premises that need to be moved and euthanized because of animal welfare concerns due to movement restrictions during a CSF outbreak.

Materials and methods: Different single-site (n=6) and multiple-site (n=5) most-likely CSF outbreak scenarios were identified using the Indiana swine premises identification data. The premises located inside the movement restriction zones were identified. Qualitative and quantitative risk models were developed to assess different strategies (movements between premises and to slaughter plants, and on-farm euthanasia) to mitigate animal welfare concerns due to movement restrictions. The time elapsed before overcrowding and/or feed shortage, epidemic duration, harvest/transition age, and the age of pigs at onset of the outbreak were estimated and used in the decision of initiating different animal-welfare mitigation strategies.

Results: The median (5th, 95th percentiles) number of swine premises under movement restrictions at the onset of a CSF outbreak was estimated to be 50 (30, 60) in the single-site and 309 (115, 315) in the multiple-site outbreak scenarios. The median number of nursery, grow-to-finish, and wean-to-finish operations under movement restrictions was 8, 30 and 9 in the single-site, and 63, 176 and 309 in the multiple-site outbreak scenarios. The median (5th, 95th percentiles) time at which either overcrowding or feed shortage emerged ranged from 18 days (4, 40) in nursery to 70 days (5, 150) in wean-to-finish premises. In the single-site outbreaks, the median numbers of the premises under movement restrictions that needed to be moved and euthanized were 20 and 12, whereas in the multiple-site outbreaks, the numbers were 139 and 94.

Conclusion: The findings could provide input to initiate mitigation strategies for animal welfare concerns due to movement restrictions. The authority could use the outcomes to estimate the resources for management (on-farm euthanasia, controlled movements, etc.) of the CSF outbreaks.

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Risk ranking of irish salmon farms based on network metrics and biosecurity evaluation

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Here we analyzed the structure and dynamics of live Atlantic salmon, Salmo salar, movements in Ireland during the years 2013-2014 using social network analysis methods, and evaluated the biosecurity practices at the farm level through the application of a detailed survey to site managers, which was used to generate a farm biosecurity score. Finally, these results were used as an input for elaborating a risk ranking, to identify sites at higher risk of disease introduction and/or spread, in order to allocate surveillance resources more efficiently, within the framework of risk-based surveillance.

Resulting site centrality measures from the network analysis (indegree, outdegree, betweenness, incloseness, and outcloseness) and biosecurity scores were analyzed using Principal Component Analysis (PCA) to reduce the number of dimensions in the data into a set of variables explaining most of the observed variability. With the resulting principal components, a K-means clustering algorithm, which partitions a dataset into k distinct non-overlapping clusters while minimizing the within-cluster variation, was carried out, setting k = 3 groups aiming to produce 3 risk categories: low, medium and high.

It is important to note, that besides live fish movement and biosecurity within the industry, there are possibly other factors involved in a site's susceptibility to disease. Hence this risk ranking is meant as a preliminary step to direct further research on the pathways or routes that contribute the most to disease occurrence on a farm.

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Modeling the transmission of Orf in order to identify the mechanisms causing persistence.

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Orf is a poxvirus causing skin disease in sheep, which persists even in closed populations. Our goal was to identify the mechanisms that lead to the persistence of Orf in sheep flocks. We developed models based on Susceptible-Infected-Recovered (SIR), Susceptible-Infected-Recovered-Susceptible (SIRS), and Susceptible-Infected-Recovered-Persistent (SIRP) modeling frameworks. For each framework, we included one model with a compartment (W) tracking Orf concentration in the environment and one model without. Each model accounted for the time dependent demography that is present in sheep flocks. We found that, with the proper choice of parameters, all the models were able to product repeating Orf outbreaks. Future experimental work will provide the data necessary to determine which model is most accurate, which will indicate the most effective control measures.

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Filling gaps in notification data: a model-based approach applied to travel related campylobacteriosis cases in New Zealand

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Abstract

Data containing notified cases of disease is often compromised by incomplete or partial information related to individual cases. In an effort to enhance the value of information from enteric disease notifications in New Zealand, this study explored the use of fully Bayesian and Multiple Imputation models to fill risk factor data gaps. As a test case, overseas travel as a risk factor for infection with campylobacteriosis has been examined.

Two methods, namely Fully Bayesian Specification (FBS) and Multiple Imputation (MI), were compared regarding predictive performance for various levels of artificially induced missingness of overseas travel status in campylobacteriosis notification data. Predictive performance of the models was assessed through Brier Score, Area Under the ROC Curve and Percent Bias of regression coefficients. Finally, the best model was selected and applied to predict missing overseas travel status of campylobacteriosis notifications in New Zealand.

While no difference was observed in the predictive performance of the FBS and MI methods at a lower rate of missingness (<10%), the FBS approach performed better than MI at a higher rate of missingness (50%, 65%, 80%). The estimated proportion (95% CI) of travel related cases was greatest in highly urban District Health Boards in Counties Manukau, Auckland and Waitemata, at 0.37 (0.12, 0.57), 0.33 (0.13, 0.55) and 0.28 (0.10, 0.49), whereas the lowest proportion was estimated for more rural West Coast, Northland and Tairawhiti DHBs at 0.02 (0.01, 0.05), 0.03 (0.01, 0.08) and 0.04 (0.01, 0.06), respectively.

We propose the use of FBS which offers a flexible approach for data augmentation particularly when the missing rate is very high and when the Missing At Random assumption holds. High rates of travel associated cases in urban regions of New Zealand predicted by this approach are plausible given the high rate of travel in these regions, including destinations with higher risk of infection. The added advantage of using a Bayesian approach is the model's prediction can be improved whenever new information becomes available.

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Role of carriers in the transmission dynamics of bighorn sheep pneumonia

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Pneumonia in bighorn sheep (Ovis canadensis) has been mainly attributed to pathogens acquired from domestic sheep and goats. However, occurrence of pneumonia in herds with no known contact with livestock, and recurrence in herds with no recent history of exposure, indicates the presence of carriers within previously exposed bighorn herds. Recurring lamb deaths in subsequent years seem to implicate a role for maternal and individual immunity against pneumonia.

We investigated the role of carrier animals, as well as maternally-derived antibodies, in bighorn sheep pneumonia. Naïve, pregnant bighorn ewes (treatment group, n = 3) were commingled with 3 previously exposed rams. All animals were monitored for presence of pneumonia pathogens (mainly Mycoplasma ovipneumoniae and leukotoxin-producing members of Pasteurellaceae), development of disease and lamb survival. As a control, 3 other naïve pregnant ewes were also monitored for presence of pneumonia pathogens and lamb survival.

Within one month of commingling, all treatment ewes acquired pneumonia pathogens from the rams. All lambs born to these ewes also developed acute pneumonia and died within 4-9 weeks of birth. Two rams and one ewe succumbed to pneumonia at 3, 8 and 10 weeks of commingling, respectively. All control ewes and lambs remained negative for pneumonia pathogens and disease.

The two treatment ewes continued to be carriers for pneumonia pathogens for the subsequent two years after the study and lambs born to them in those years also succumbed to pneumonia within 4-10 weeks of birth. Antibody titers against pneumonia pathogens in the surviving ram and two ewes seemed adequate enough to be 'protective' (no apparent respiratory disease). The lambs, however, were either born with comparatively low titers or titers dropped dramatically within the first 2-10 weeks of age, making them susceptible to the disease.

Thus, we have established that bighorns previously exposed to pneumonia become carriers of the pathogens and sources of infection for naïve animals, including newborn lambs. Our findings also suggest an important role for maternally-derived antibodies in resistance to pneumonia in bighorn lambs.

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Developing sampling guidelines for oral fluid-based PRRSV surveillance

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Purpose

Oral fluids (OF) are a convenient surveillance sample because they are easily collected and can be tested for nucleic acids and/or antibodies for PRRSV and a variety of pathogens. We are currently developing statistically-based guidelines for sample size, frequency, and location.

Methods

Two studies were conducted to map the spatiotemporal aspects of PRRSV infection and further the development of oral fluid sampling guidelines. Study 1 - In one WTF barn on each of 10 production sites, OF samples were collected from 6 equidistant pens (~25 pigs per pen) every 2 weeks for 18 weeks. Study 2 - In 3 WTF barns on one finishing site, OF samples were collected weekly from every occupied pen (108 pens; ~25 pigs per pen) for 8 weeks. OF samples were randomized and then tested for PRRSV RNA, IgG, and IgA. To date, statistical analyses have been done to examine spatial autocorrelation, compare detection based on systematic spatial vs random sampling, and compare sampling

from the same pens vs alternate pens at each time point. Further analyses are ongoing.
Results
Analyses showed that the disease status of a pen in a barn was highly influenced by the status of other pens in the same barn, i.e., the presence of ≥ 1 positive pens increased the odds of detecting another positive pen.
Analysis showed that systematic spatial sampling was as good as or better than random sampling. Sampling the same pens at each time point was more effective than changing the pens sampled at each time point.
Conclusions
Spatial autocorrelation has previously not been quantified at the barn level and has important implications for surveillance. Sample size calculations are in progress, but frequency of sampling is more important than sample size. That is, fewer samples collected at regular 2-week intervals are more useful that more samples collected at long intervals.

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Detecting human brucellosis in rural Uganda: Comparison of a commercial lateral flow assay with microagglutination on sera from high-risk subjects

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Purpose: As part of a larger cross-sectional project to determine the seroprevalence of brucellosis in cattle, goats, and humans in southwestern Uganda, a study was conducted to compare results from a commercially available lateral flow assay (LFA) for detection of *Brucella*-specific IgG and IgM to *Brucella* microagglutination test (BMAT) on sera collected from subjects on 70 cattle farms, and to identify factors associated with LFA and BMAT results, and discordant findings between both tests.
Methods: Sera were collected from human subjects, and blood and milk were collected from livestock and tested for brucellosis. The LFA was used in the field within 12 hrs of sample collection. A subset of frozen sera were tested by the CDC with the BMAT for IgG and total antibodies (AB). Data on subjects, including age, gender, history of fever, and known risk factors for brucellosis, and household-level human and animal health and management data were collected at sampling. Associations between risk factors and test results were evaluated using multivariable mixed models with farm as a random effect.
Results: A total of 236 serum samples were collected from 174 men and 61 women, of which 29 were positive by the LFA (26 IgG-positive, two IgG- and IgM-positive, one IgM-positive). Of 189 sera tested by the CDC, 169 were negative (IgG negative, AB <1:20), 19 were inconclusive (IgG < 1:20, AB 1:20 - 1:80), and one was positive for *Brucella* (IgG = 1:160, AB = 1:320): this subject was IgG-positive and IgM-negative by the LFA. Based on multivariable analyses, risk factors for seropositivity included increasing subject age and household size. Concordance between the LFA and BMAT was 87.2% and 87.3% for IgG and AB, respectively, with 24 BMAT-negative/LFA-positive tests for both IgG and AB. A household history of consuming raw milk and brucellosis seropositive cattle were associated with discordant results in multivariable analyses, whereas household history of arthralgia symptoms was associated with concordant results.
Conclusions: The LFA was as effective as the BMAT at identifying negative subjects, but any test results should be interpreted in context of subject and household risk factors for brucellosis.

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Comparative study of diagnostic tests for Tuberculosis in cattle

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Bovine tuberculosis is an economically important and major zoonotic disease. Early diagnosis is required to implement effective control strategies. Commonly available diagnostic tests include single intra dermal (SID) test an in-vivo test and, interferon gamma (INF-γ) assay, isolation of the causative agent and detection of antibodies by ELISA. The present study reports the comparison of diagnostic tests for the diagnosis of tuberculosis in a dairy herd near Bangalore, south India, with the history of tuberculin reactors in the previous year which were subsequently removed. Forty five, Holstein Friesian cross bred cows were screened by SID test, INF-γ assay, ELISA to detect antibodies and isolation of the organisms from milk and nasal swabs. The results revealed that eight of 45 cows reacting to SID test; ten cows positive by INF-γ assay and none to antibody ELISA. Six SID reactors were also positive by INF-γ assay. Twelve animals which were positive by SID test and/or INF-γ assay were subjected for mycobacterial isolation. On culturing on to LJ media (without glycerol) and middle brook 7H9 media, none of the milk samples yielded mycobacterial growth while, two of the twelve nasal swabs tested yielding growth suggestive of mycobacteria, further they were confirmed to Mycobacterium tuberculosis complex by multiplex PCR with a specific amplicon size of 245 bp, but did not yield 500 bp product specific for M.bovis. Cell mediated immune response based tests performed better than those targeting antibody detection and isolation. Between the tests, INF-γ assay (22.22%) detected more positives indicating its better sensitivity than SID test (17.77%). Prevalence of tuberculosis in this farm was estimated to be 26.67 per cent. It can be concluded that though IFN- γ assay appeared to have an edge over other tests, no single test is able to detect all positives, and combination of tests is required to diagnose tuberculosis in cattle.

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Comparative evaluation of blood based Lateral Flow Assay with other serological tests and Polymerase Chain Reaction for the diagnosis of Brucellosis in livestock

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Brucellosis is a zoonotic and economically important disease. The present study reports the evaluation of blood based Lateral Flow Assay (LFA) with other serological tests and Polymerase Chain Reaction (PCR) for the diagnosis of Brucellosis in different livestock species. Materials

collected include 792 sera/blood samples; 153 cattle, 55 buffalo, 140 sheep, 219 goats and 225 pigs from organized/unorganized farms from southern peninsular India, with the history of repeat breeding, retention of placenta and abortion. The methods employed were Rose Bengal Plate Test (RBPT), Serum Agglutination Test (SAT), indirect ELISA (iELISA), serum and blood based LFA and PCR. The seroprevalence of brucellosis in cattle, buffalo, sheep, goat and pigs was found to be 7.84, 3.63, 12.85, 2.73 and 39.11 per cent by RBPT; 11.1, 9.09, 16.42, 5.93 and 46.6 per cent by iELISA; 5.88, 3.63, 8.57, 2.73 and 31.1 per cent by LFA (blood) and 7.18, 3.63, 10.71, 3.19 and 32.88 per cent by LFA (serum). Five of 14 bovine samples and 15 out of 26 sheep and goat samples were found positive by SAT. Relative specificity for all the species was found to be greater than 99 per cent for LFA (blood) and 92 per cent for LFA (serum) whereas relative sensitivity for all the species ranged from 50 to 83.33 per cent by LFA (blood) and 53.85 to 84.09 per cent by LFA (serum). Diagnostic accuracy ranged from 84 to 99 per cent by LFA (blood) and 86 to 98 per cent by LFA (serum) for all the species compared to RBPT and ELISA respectively. Higher seroprevalence of brucellosis in pigs was traced back to introduction of an infected boar for breeding purpose from a local market without any prior testing. Seropositivity was highest by iELISA followed by RBPT, LFA and SAT. Genus specific PCR for 55 bovine, 47 sheep and goat and 75 pig serum/plasma samples yielded a specific amplicon of 223 bp from five (9.09%), nine (19.14%) and 38 (50.66%) respectively. It is concluded that blood based LFA can be used as an alternative pen side diagnostic test for screening of brucellosis among multiple species and recommended for adaptation on-farm, slaughter, market, clinical and pre-purchase surveillance of brucellosis in the country.

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Detection of Dichelobacter nodosus and Fusobacterium necrophorum from footrot of sheep and goats in Andhra Pradesh: Southern India.

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Purpose :
To investigate the lameness in sheep and goats, study was undertaken to identify the Dichelobacter nodosus and Fusobacterium necrophorum from foot lesions of sheep and goat of Andhra Pradesh, Southern India, using PCR.
Methods:
A PCR assay targeting the 16S rRNA gene of D. nodosus and lkt A gene of F. necrophorum was used to test the 107 field samples collected from inter digital spaces of sheep and goat with typical footrot lesions. Further, multiplex PCR was used to identify the prevailing serogoups of D.nodosus.
Results:
The nucleotide sequences of 16S rRNA and lkt A amplicons of D. nodosus and F. necrophorum revealed 98% and 99% homology with corresponding published sequences. The partial sequences of 16S rRNA gene of D. Nodosus and lktA gene of F.necrophorum were submitted to the GenBank (JN008724, KP226805 & KP226806). Out of 107 foot swabs collected, 74 were from sheep and 33 were from goats. Among the 74 sheep samples screened, 34 (45.94%) were tested positive for D.nodosus and 16 (21.62%) were positive for F.necrophorum whereas 15 (20.27%) were positive to both the bacteria. The combined presence of F.necrophorum with D.nodosus supports the hypothesis that the synergistic interaction between the two organism cause footrot in sheep. Among 33 goat samples, 10(30.30%) were positive for D.nodosus and, 18(54.54%) were positive for F.necrophorum. A total of 10(30.30%) goat samples were positive for both D.nodosus and F.necrophorum. The predominance of F.necrophorum in the goats in present study supports the argument that F.necrophorum alone might cause the footrot in goats. Results of multiplex PCR reveald presence of A,B,C and I serogrops of D.nodosus.
Conclusions:
D.nodosus and F.necrophorum were identified from footrot lesions of sheep and goats in tropical climate of Andhra Pradesh. Multiples serogroups A,B,C and I of D.nodosus were recorded. Serogroping of F.necrophorum need to be carried over for decision making for long term management of footrot in sheep and goats in the region. Further investigations are required to understand the survivability of D.nodosus in the unusual niche of present tropical climate.

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Experiential learning: The Farm to Table Study Program as a case study

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Experiential learning, or learning through structured experience involving reflection and analysis, can lead to multiple positive learning outcomes, including deeper understanding of content material as well as development of group leadership skills. The Farm to Table Study Program, offered annually for the past seven years in an exporting Latin American country through collaboration between the University of Minnesota, Ohio State University, and a local university, provides participants the opportunity to explore firsthand animal food production systems from farm-to-table. Site visits along the food supply chain are focused on production, international trade standards, and emerging issues in food safety and animal and public health. Direct discussions with key government and private sector leaders and interactive multi-sectorial and cross-culture group activities are designed to integrate knowledge and skills. This program is an example of the type of systems-based experiential learning needed by the next generation of public health and food systems professionals.

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Mark Gearhart Memorial Award, Salon A/B/C/D Performance and carcass characteristics of commercial feedlot cattle from a study of vaccine and direct-fed microbial effects on *Escherichia coli* O157:H7 fecal shedding

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The objective of this study was to quantify cattle performance and carcass characteristics associated with administration of a siderophore receptor and porin proteins-based vaccine (VAC) and a direct-fed microbial (DFM), which were originally evaluated for their impact on *Escherichia coli* O157:H7 fecal shedding in a commercial feedlot population. Cattle (n = 17,148) were randomly allocated into 40 pens grouped by allocation dates into 10 complete blocks; pens within block were randomly allocated to control, VAC, DFM, or VAC + DFM treatment groups in a 2 × 2 factorial design. The DFM (Bovamine) was fed daily at the labeled dose of 106 cfu/animal of *Lactobacillus acidophilus* for the duration of the intervention period (mean = 86.6 d). The VAC cattle were vaccinated on Days 0 and 21 whereas unvaccinated cattle were not given a placebo or rehandled on Day 21. Data were analyzed using general and generalized linear mixed models that accounted for the study design. Main effects of DFM and VAC are reported as there were no significant treatment interactions for any of the outcomes evaluated. Vaccinated cattle had lower total weight gain ($P < 0.01$), ADG ($P = 0.03$), and cumulative DMI during the intervention period ($P < 0.01$) compared with unvaccinated cattle, whereas the DFM increased total weight gain ($P = 0.03$) and G:F ($P = 0.05$) during the intervention period. Daily DMI was decreased ($P < 0.01$) in vaccinated pens compared with unvaccinated pens during a 5-d period immediately following revaccination. After the intervention period was completed, cattle were sorted following the standard operating procedure for the feedlot and all cattle were fed the DFM from that point until harvest. Each steer was individually identified through harvest. At harvest, vaccinated cattle had more total days on feed ($P < 0.01$) with a larger HCW ($P = 0.01$) than nonvaccinated cattle, whereas cattle not fed the DFM during the intervention period had a significantly larger HCW ($P < 0.01$) than those fed the DFM during the intervention period. We conclude that the use of these DFM and vaccine products have differential and independent effects on cattle performance and carcass characteristics in a commercial feedlot setting.

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Understanding PEDV transmission by live haul transport at swine lairage facilities

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Previous studies showed live animal transport to harvest plants is a potential route of transmission during Porcine Epidemic Diarrhea Virus (PEDV) outbreaks. We developed a model to replicate the process of unloading pigs into lairage. The trailer was modeled using an aluminum sheet; a plastic tub filled with manure and shavings simulated the unloading dock. A foot contact event was mimicked using a plastic boot to step from model dock onto model trailer. Various conditions on dock (temperature, UV light, scraping) and trailer (temperature, humidity) were tested. PEDV was detected pre- and post-contamination using real-time polymerase chain reaction (RT-PCR). In trial 1 we investigated the impact of trailer temperature on PEDV persistence over 1 hour. Trial 2 focused on dock conditions including scraping, temperature, UV light intensity and time post-contamination on PEDV contamination in the trailer at 1 hour post contact. Under conditions of the study, transmission of PEDV was highly efficient as PEDV RT-PCR CT values were similar on the dock, at time 0 and at 1 hour post contact on the trailer. UV light on the dock and dock temperature did not influence the rate of PEDV transmission. Removing gross contamination (scraping) of the dock eliminated transmission by 10 minutes' post scraping. This model confirms that lairage is a significant risk for dissemination of PEDV between swine production sites and that simple procedures such as manual removal of gross contamination could minimize the risk of PEDV transmission in lairage.

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Developing sampling guidelines for PEDV surveillance

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Oral fluids (OF) are useful for surveillance because they are easily collected and can be tested for nucleic acids and/or antibodies to determine the infection status of the individuals contributing to the samples.[1] Assays for testing swine OF specimens have been available at veterinary diagnostic laboratories since 2010 and pen-based OF sampling has become common practice for monitoring a variety of endemic pathogens of swine, e.g., PEDV, PRRSV, PCV2, IAV and others.

PEDV was identified in the US in April 2013 by diagnosticians at the ISU-VDL.[2] Shortly thereafter, a PEDV RT-PCR became available for routine testing and ~39,000 OF samples and 47,000 fecal samples were tested between May 2013 - November 2014. A PEDV serum IgG indirect ELISA became available for routine testing in September 2014.[3] This ELISA is also available on request for the detection of IgA and/or IgG in OF, colostrum, and milk.

The purpose of the study reported herein was to describe the spatiotemporal pattern of PEDV circulation in the field and to develop sampling guidelines. OF samples were collected from 36 pens (~25 pigs per pen) in 3 wean-to-finish barns on 3 sites for 8 weeks beginning one week post placement. ~2,916 individual OF samples (108 pens per site x 9 sampling points x 3 sites) were tested for virus (RT-PCR) and antibody (IgG, IgA).

Analyses performed to date confirm the utility of OF in surveillance and suggest a high degree of variability in the circulation of PEDV within

and between sites, i.e., the distribution of positive pens and disease progression was unique to each individual barn. Assessment of spatiotemporal patterns supports the conclusion that all barns must be sampled in order to accurately establish disease status of a site.

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Mental health and wellness in veterinarian and agricultural producers in Ontario, Canada

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Veterinarians in Australia experience levels of burnout and depression higher than those of the general population, and in the United Kingdom (UK), 26% of veterinarians studied were afflicted with anxiety. In the UK, veterinarians are reported to have a risk of dying by suicide that is 5-7 times higher than the general population.

Mental health needs amongst agricultural producers are also being increasingly recognized, particularly in the wake of large-scale animal disease outbreaks, mass animal depopulations, and extreme weather events. By way of example, after the 2001 Foot and Mouth outbreak in the UK, much higher rates of psychological illness were observed amongst producers in affected areas than in non-affected areas; it was also associated with the degree of animal culling and movement restrictions imposed. Producers in several countries worldwide report a wide array of significant occupational stresses. Further complicating the issue, reports of producers' help-seeking behaviours indicate this population is difficult to reach effectively.

It is important to uncover the factors associated with mental health issues in veterinarians and producers, in order to help limit the impact of the occupational stresses they experience. To this end, a cross-sectional pilot study of veterinarians in Ontario, Canada, began in summer 2015 to determine the prevalence of, and factors associated with, depression, anxiety, burnout, compassion fatigue and resilience. A second pilot study of producers from a variety of agricultural industries will be initiated in fall 2015 to investigate these outcomes, and explore occupational stresses and help-seeking attitudes and behaviours in this population.

Preliminary analyses of the prevalence of mental health outcomes in veterinarians and agricultural producers, as well as associated risk and preventative factors, will be presented.

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Comparison of Johne's disease prevalence on organic and conventional dairy farms in Pennsylvania

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Johne's disease (JD) affects approximately 70% of all US dairies, and can be a cause of great economic loss to dairy producers. To qualify for the label "organic", certain restrictions on management practices may predispose for the transmission of JD on the farm. The objectives were: 1- to compare JD prevalence between Pennsylvania organic and conventional dairy farms, and 2- to identify risk factors associated with differences in JD prevalence. A JD milk ELISA was performed on individual milk samples from each lactating cow in the study herds. Information regarding management practices was collected during a farm visit. Overall herd prevalence, and within-herd prevalence were compared between groups. Logistic regression was used to identify risk factors associated with differences in JD prevalence between groups. A total of 2,739 cows from 50 herds (26 organic and 24 conventional) were included in the study. Median herd size was 58 (range 20-114 cows) for conventional farms, and 39 (range 20-211 cows) for organic farms. Of all the farms included in the study, 27/50 (54%) were positive, with 14/24 (58%) positive conventional farms and 13/26 (50%) positive organic farms. From the conventional farms, 25/1,506 (2%) cows were positive, compared with 28/1,233 (2%) cows from organic herds. After adjusting for herd size, there was no significant difference in between-herd prevalence (P=0.55). Although within-herd prevalence was slightly higher (+2.4%) in JD positive organic herds (range 1-15.4%) compared to JD positive conventional herds (1-5%) the difference was not statistically significant (P=0.06). Risk factors are currently being analyzed. Preliminary results indicate that there is no difference in JD prevalence between Pennsylvania organic and conventional dairy farms.

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Meta-analysis of the effects of laidlomycin propionate or monensin sodium on performance, health, and carcass outcomes in finishing steers in North America

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According to a 2011 national survey, over 90% percent of U.S. feedlots over 1,000 head capacity include an ionophore, such as monensin sodium (Rumensin®) or laidlomycin propionate (Cattlyst®), as part of their nutritional management program. Ionophores are fed to finishing cattle to improve feed efficiency and weight gain. The objective of this study was to describe the effects of feeding laidlomycin propionate or monensin sodium, alone or in combination with antibiotics, at commercially approved dosages, on growth performance (average daily gain (ADG),

feed:gain (F:G), and dry matter intake (DMI)), health (overall and cause-specific mortality) and carcass characteristics (hot carcass weight (HCW), and liver abscesses) in finishing steers in North America, using a systematic review and meta-analysis. Data extracted from peer-reviewed articles and from industry reports were incorporated into meta-analyses models to compute summary intervention estimates for different outcomes of interest. Pooled mean differences for continuous outcomes and odds ratios for dichotomous outcomes, and their 95% confidence intervals, were obtained from random effects meta-analyses models using the DerSirmonian and Laird method. There was evidence for significant (P-values < 0.05) beneficial effects of feeding laidlomycin propionate to finishing steers compared to feeding monensin sodium on performance (ADG, DMI and F:G) and carcass traits (HCW). Mortality outcomes were not significantly different between treatments, but the occurrence of liver abscesses was significantly greater in steers fed laidlomycin propionate compared to steers fed monensin sodium. However, there was evidence of significant heterogeneity among studies. Meta-regression models evaluating variables such as pen size or production setting failed to explain the heterogeneity for most outcomes of interest. This systematic and quantitative summary of the effects of this important class of feed additives in the feedlot industry is unique and provides useful information on relevant outcomes that are of economic importance to end-users.

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Cluster analysis of Campylobacter isolates obtained from beef cattle, dairy cattle, swine, and mammalian wildlife on Southern Ontario farms

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Purpose:
The objectives of this study were to identify the potential sharing of Campylobacter subtypes between livestock and wildlife based on molecular subtyping and assess if mammalian wildlife/livestock carry host-specific subtypes of Campylobacter jejuni.

Methods: Using data collected from a cross-sectional study of twenty-five farms in 2010, we assessed clustering of molecular subtypes of C. jejuni based on a Campylobacter-specific 40 gene comparative genomic fingerprinting assay (CGF40), using UPGMA analysis, multiple correspondence analysis, and exact logistic regression to determine if and what genes identify wildlife and livestock subtypes in our study population.

Results:A total of 33 livestock and 26 wildlife C. jejuni isolates were subtyped using CGF40, only a single subtype was seen in both wildlife and livestock isolates. Dendrogram analysis, based on UPGMA, showed a single cluster containing all but 2 wildlife subtypes, while the remaining branches contained the majority of livestock subtypes. Furthermore, multiple correspondence analysis agreed with the dendrogram by also showing clear differentiation between livestock and wildlife subtypes. Exact logistic regression conducted gene-by-gene revealed 15 genes that were predictive of whether a subtype was of wildlife or livestock isolate origin. For most of these genes, their presence was predictive of an isolate being of livestock origin.

Conclusions:
We concluded from the evidence gained from dendrogram analysis, multiple correspondence analysis, and exact logistic regression that mammalian wildlife typically carry subtypes of C. jejuni that are distinct from those carried by livestock.

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Isolation and characterization of Salmonella spp. from captive wild animals

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Salmonellosis is a zoonotic disease and a wide variety of Salmonella serotypes have been detected in wild animals throughout the world. The present study reports the prevalence of Salmonella in captive wild animals in a Biological Park, Bangalore, south India. One hundred fifty five clinical samples viz., 107 mammalian fecal samples, 28 reptiles cloacal samples, 10 water samples, 10 feed samples were collected during a one year study period from April, 2013 to April, 2014. The methods employed include culturing of test samples in Rappaport Vassiliadis enrichment broth and plating on Brilliant green agar and Xylose Lysine Deoxycholate agar. Presumptive colonies were then characterized by biotyping. Results revealed variations in citrate utilization, ornithine decarboxylation, motility, H2S production and six of the isolates showing urease positive reaction. Nineteen biochemically confirmed isolates were further subjected for genus specific polymerase chain reaction (PCR) targetting invA gene. Only eleven of the 19 isolates revealed the presence of invA gene. Sequencing and phylogenetic analysis revealed that the isolates belonged to Salmonella enterica species. Antibiogram profile of all 19 isolates revealed 100 per cent sensitivity to gentamicin, 78.94 per cent to ciprofloxacin and chloramphenicol. Prevalence rate of Salmonella in captive wild animals and reptiles was estimated to 12.25 per cent (19/155), which may possibly maintain the carrier status and excrete Salmonellae. Strict biosecurity measures were implemented in the biological park to prevent any possible outbreaks. It was concluded that isolation, biotyping and PCR targeting invA gene can be used for the detection of Salmonella in wild animal species.

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Following Salmonella Heidelberg through a poultry integrator.

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Salmonella Heidelberg continues to be a food safety and human health concern. This study examined Salmonella Heidelberg presence throughout a poultry production process, from broiler breeder farms to processing plant. The epidemiological assessment of Salmonella Heidelberg within the process along with Salmonella phenotype and genotype evaluation helps to modulate field interventions such as custom vaccine applications in real time. The present comprehensive study provides information on the variability within Salmonella Heidelberg isolates found throughout the

integrator process. Phenotypic evaluation was based on growth characteristics, motility, biofilm formation, pH tolerance and general chemistries using conventional microbiology techniques and phenotype microarrays. Whole genome sequencing identified variation at the genetic level that includes large deletions, multi drug resistant plasmids and Single Nucleotide Polymorphisms (SNPs) in several biological function categories, such as metabolism, pathogenicity, biofilm formation and transport. In addition, we can compare field isolates to current administered vaccines to identify any drift. Ultimately, this study can provide insight into points of intervention and improvements to biosecurity and vaccine selection and administration.

Conclusions:

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T lymphocytes induced after infection with a single PRRSV strain recognize epitopes processed from highly diverse PRRSV strains

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Live attenuated virus vaccines for porcine reproductive and respiratory syndrome virus (PRRSV) induce partial protection with moderate reductions in viremia and clinical disease, even before the appearance of weakly neutralizing antibodies in pigs, suggesting the contribution of cell-mediated immunity (CMI). However, PRRSV-specific CMI responses need to be defined before being considered as a broadly protective mechanism against diverse PRRSVs. Herein, the breadth of CMI responses in pigs at different ages were analyzed against various PRRSV strains and proteins using T cells induced by each of two type II PRRSV strains. The hypothesis was that PRRSV-specific CMI responses induced by a PRRSV strain can recognize diverse isolates, overcoming limited homologous protection due to antigenic variations. An enzyme-linked immune-spot (ELISpot) assay using 12 PRRSV strains and overlapping-peptides encompassing all open reading frames of PRRSVSD23983 was utilized to evaluate CMI responses in pigs (sow, 8-week-old pigs and 2-week-old piglets) infected with SD23983 or VR-2385 strain.

T cell responses from all sows (n=3) recognized antigenically diverse PRRSVs (>100 spot-forming unit), as well as multiple proteins of homologous PRRSV (SD23983 strain). Similarly, seven of ten 8-week-old pigs showed broad CMI responses against various PRRSV strains after challenged with VR-2385 strain. In contrast, only two of nine 14-day-old piglets intrauterine-challenged with SD23983 strain had strong and broad CMI responses. It is noteworthy that neutralizing antibody responses in the sows and piglets with broad CMI responses are only specific to the homologous PRRSV strain. These results suggest that the induction of CMI responses that recognize diverse isolates may contribute to overcoming the problem of suboptimal heterologous protection by conventional vaccines.

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Measuring bovine γδ T cell function at the site of *Mycobacterium bovis* infection

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The causative agent of tuberculosis in cattle is *Mycobacterium bovis.* The characteristic lesions of bovine tuberculosis are well-organized pulmonary granulomas. γδ T cells are a unique subset of nonconventional T cells that play major roles in both the innate and adaptive arms of the immune system. Bovine γδ T cells have the capacity for multiple immune functions during infection with *M. bovis;* however, specific γδ T cell responses *in vivo* at the site of infection remain unclear. Using a bovine model of experimentally induced *M. bovis* infection, samples were collected from representative granulomatous lesions in the lungs and mediastinal lymph nodes at approximately 3 months after infection. Tissue sections were preserved onto slides by formalin fixation and paraffin embedding. mRNA transcripts for γδ T cells, IFN-γ, IL-17, and IL-10 were microscopically evaluated within the granulomas using an *in situ* hybridization system, RNAScope (Advanced Cell Diagnostics Inc.). Granuloma maturity was then staged I-IV based upon lesion size, cellular composition, mineralization, fibrosis, and extent of necrosis. Cytokine expression by γδ T cells at the site of infection was scored relative to granuloma maturity. This novel ISH assay revealed evidence that bovine γδ T cells accumulate in all stages of maturation within the granulomas. However, expression of key cytokines by the γδ T cells varied between stages of lesions. γδ T cells have been hypothesized to produce significant concentrations of IFN-γ within developing granulomas. However, we emonstrate here that γδ T cells are not a predominant source of IFN-γ *in situ* at this time-point after infection. Similar to IFN-γ, IL-17 was also rarely expressed by γδ T cells at this chronic phase of infection. Interestingly, however, γδ T cells were determined to express significant levels of IL-10 within late-stage granulomas. Increased amounts of IL-10 indicate anti-inflammatory properties that are characteristic of mature lesions. To our knowledge, this is one of the first reports of γδ T cells expressing IL-10 *in vivo* in the bovine, and more importantly, the first demonstration of γδ T cells producing IL-10 at the site of *M. bovis* infection.

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Characterization of recombinant PRRSV nsp1beta mutants in a nursery pig model

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Porcine reproductive and respiratory syndrome (PRRS) causes major economic losses to the swine industry since its appearance in early 1990s, reported both in the US and globally. PRRSV infection dampens the host innate immune response in pigs of all ages. PRRSV nonstructural protein 1β (nsp1β) is a strong innate immune antagonist, and a conserved GKYLQRRLQ motif of nsp1β was previously identified to play an important role in the suppression of type I interferon response in vitro. In this study, immunogenicity of three recombinant viruses (vR128A, vR129A, and vRR129AA) generated by mutating key residues in highly conserved motif, 123GKYLQRRLQ131, was analyzed in a nursery pig model. Our results indicated that pigs infected with nsp1β mutants, especially vRR129AA, had increased IFN-α production in the lungs during early time points post-infection, which was correlated with an increased innate NK cell function. Furthermore, augmented innate response was consistent with increased production of IFN-γ in those mutated viruses-infected pigs. These data demonstrate that R128 and R129 residues are

critical for nsp1β function in vivo, and modifying these key residues in the GKYLQRRLQ motif improved the innate and adaptive immune responses in infected animals. This project is supported by USDA-AFRI; Kansas State University; and The Ohio State University research funds.

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Reduced antigen-specific antibody levels in cows naturally infected with bovine leukemia virus

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Bovine leukemia virus (BLV) is a retrovirus that is highly prevalent within U.S. dairy herds. 83% of dairy herds are BLV-infected, and the within-herd infection rate is estimated to be as high as 46%. Evidence suggests that BLV causes immune suppression in infected cows, which could lead to decreased vaccine responses and increased risk of infectious diseases. Measuring the effects of BLV induced immune suppression is essential to estimate a true economic burden for BLV in dairy herds and push producers towards BLV control and eradication. We have found that B cells from BLV-infected cows have lower surface expression of MHCII and a significantly smaller percentage expressing CD45R0, which suggests impairment in the development of memory B cells. To more clearly assess the humoral memory immune response, healthy cows and cows naturally infected with BLV received a boost vaccination with BoviShield Gold FP5 L5 HB. Serum was collected over a 28-day time course post-vaccination and antigen-specific neutralizing antibody, IgM, IgG1 and IgG2 levels were measured. BLV-infected cows exhibited significantly lower antibody titers compared to healthy cows, strongly suggesting that the humoral memory immune response is impaired in BLV-infected cows.

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Identification of immunodominant B cell epitopes in the C. pecorum proteome

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Laboratory methods for identification of B-cell epitopes are time-consuming and labor-intensive, and epitope prediction algorithms from primary protein sequences are highly unreliable. Using extensive data on confirmed epitope and non-epitope regions of the *Chlamydia* spp. proteome, we improved *in silico* B cell epitope prediction to 87% specificity at 80% sensitivity. This improved prediction accuracy is based on the IUPred-L algorithm for calculating protein disorder tendency, a fundamental property of natural B cell epitopes, as well as an overall property of immunodominant proteins. For the current investigation, we sought to identify immunodominant B-cell epitopes of *C. pecorum* by exclusive *in silico* pre-screening prior to testing the antibody reactivity of these predicted B cell epitopes. By ranking all proteins of the whole *C. pecorum* proteome for high protein disorder tendency, followed by secondary ranking of the highest scoring proteins for highest divergence from other chlamydial species, we predicted and selected from ten proteins 4-10 putative B cell epitopes each with very high potential for high, and highly specific *C. pecorum,* reactivity. These epitopes were chemically synthesized as N-terminal biotinylated 16-40aa peptide antigens, captured onto streptavidin-coated microtiter plates, and reactivity was tested with sera of cows and calves naturally infected with *C. pecorum*. This approach identified 1-7 strongly antibody-reactive peptide antigens from nine of the ten proteins tested. These B cell epitope peptides are highly suitable for species-specific detection of anti-*C. pecorum* antibodies. Among the advantages of this current method are (i) high inherent sensitivity due to identification of dominant B-cell epitopes; (ii) high specificity by selection of highly divergent epitope regions of these proteins; and (iii) minimal wet lab testing requirement for identification of these peptide antigens. This proposed approach offers promise for identifying suitable peptide antigens of other pathogens for diagnostic uses as well as candidate B cell epitopes for subunit vaccines.

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A synthetic biodegradable microsphere vaccine of femtomole-dosed peptide antigens protects better against Chlamydia abortus than previous infection

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Successful vaccination against *Chlamydia* spp. has remained elusive, largely due to a lack of vaccine platforms for the required Th1 immunization. Modeling of T helper cell immunity indicates that Th1 immunity requires antigen concentrations that are orders of magnitude lower than those required for Th2 immunity and antibody production. We hypothesized that the *C. abortus* vaccine candidate proteins that we identified earlier, DnaX2, GatA, GatC, Pmp17G, and Pbp3, mediated protection in an A/J mouse model of *C. abortus* lung infection if administered each at 11-90 femtoMoles per mouse. This immunization significantly protected the mice from lethal challenge with 10^8 *C. abortus* organisms. Additional experiments proved that particulate delivery of antigens was required for optimum immunity. We further hypothesized that i) 20-mer peptides overlapping by 10 amino acids could substitute for the whole protein antigens (249 peptides total); ii) that release of peptides and adjuvant from 1-10 µm microspheres would enable controlled generation of Th1 immunity; and iii) that inhibition of apoptosis could suppress the inflammatory Th17 response and enhance a protective Th1 response. Solutions of peptide antigens with biodegradable poly (lactide-co-glycolide) copolymer (PLGA) and the block copolymer adjuvant Pluronic L121, with or without the apoptosis inhibitor Q-VD-OPH, were spray-dried to 2 µm microspheres, which were administered subcutaneously or intranasally at 10 µg per mouse in a 129S6 mouse model of *C. abortus* lung infection. A dose of 2 femtoMoles each peptide per mouse significantly reduced the disease but failed to effectively eliminate chlamydiae from the lungs. In contrast, a dose of 0.2 femtoMoles each peptide along with 0.2 µg Q-VD-OPH per mouse generated highly significant protection against *C. abortus*. In conclusion, we have developed a fully synthetic biodegradable microsphere vaccine for controlled release of adjuvant and ultralow doses of peptide antigens. This vaccine platform can be used for real-life vaccines as well as a tool to model chlamydial immunopathogenesis by manipulating the vaccine immune response.

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15-F2t-Isoprostane concentrations correlate with oxidant status in lactating dairy cattle with acute coliform mastitis.

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Severe mammary tissue damage during acute bovine coliform mastitis is partially due to oxidative stress. Although considered a gold standard biomarker in some human conditions, the utility of 15-F2t-Isoprostanes (15-F2t-Isop) in detecting oxidative stress in dairy cattle has not been validated. The hypothesis for this study was that concentrations of 15-F2t-Isop in plasma, urine, and milk correlate with changes in oxidant status during severe bovine coliform mastitis. To address this hypothesis, lactating Holstein-Friesian dairy cows in their 3rd - 6th lactation with acute coliform mastitis (n = 4) and matched controls (n = 4) were enrolled into mastitis and control groups, respectively. Measures of inflammatory status, oxidant status, and redox status in plasma and milk samples were quantified using commercial assays. Plasma, urine, and milk 15-F2t-Isop were quantified by liquid chromatography/tandem mass spectrometry (LC-MS/MS) and commercial ELISA assays. Data were analyzed by Wilcoxon rank sum tests ($\alpha = 0.05$). Plasma 15-F2t-Isop quantified by LC-MS/MS positively predicted systemic oxidant status. Urine 15-F2t-Isop quantified by LC-MS/MS did not predict the systemic oxidant status but correlated with redox status parameters. Milk 15-F2t-Isop quantified by LC-MS/MS was not predictive of local oxidant status. Total 15-F2t-Isop in milk quantified by a commercial ELISA was predictive of oxidant status in milk. In conclusion, free plasma 15-F2t-Isop quantified by LC-MS/MS and total milk 15-F2t-Isop quantified by ELISA are accurate biomarkers of systemic and mammary gland oxidant status, respectively. Establishing reference intervals for free and total 15-F2t-Isops for evaluating oxidative stress in dairy cows should currently be based on the LC-MS/MS method.

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Regulation of host immune gene expression by Torque Teno Sus Virus1 (TTSuV1) non-structural proteins

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Recent studies suggest that Torque Teno Sus Viruses (TTSuV's) are strongly associated with pathogenic swine viruses, such as the porcine reproductive and respiratory disease syndrome virus (PRRSV) and porcine circovirus strain 2 (PCV2). To test the hypothesis that TTSuV1 can potentiate other viral agents by causing immune suppression, we determined whether TTSuV1's non-structural proteins regulate key host immune factors. Immortalized swine alveolar macrophage cells, 3D4/31, were transfected with plasmids expressing the non-structural proteins encoded by open reading frames (ORF) 1 or 2. Samples were collected at 12, 24 and 48 hours post-transfection. The differential expression of a panel of immune factors, including but not limited to RIG-1, TLR-3, TLR-7, TLR-8, TLR-9, type I and II interferons, Mx1, Mx2, OAS1, RNAseL, PKR, SOCS1, IL-3, IL-10, PD-1, IL-13 TNFα, and TRAIL was studied by quantitative real-time PCR. The five reference controls included β actin, β2M1, GAPDH, HPRT and TBP-1. At 12 and 24 hours, IFN-β, Mx-1, the interferon induced genes, NLRP3, DAI/ZBP1, LGP2/DHX58, IL-4, IL-10 and IL-13 and MAD5 were down regulated by both ORF1 and 2, whereas IL-1β, IL-6 and RIG-1 were upregulated. PD-1 and TLR-3 were upregulated in response to ORF-1 whereas down regulated by ORF-2. However, other significant changes were not evident at 48 hours. Our in-vitro data suggests that the TTSuV1 non-structural proteins are predominantly immunosuppressive during very early infection and the early immune-suppression could facilitate the establishment of chronic TTSuV infections in the host.

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Nsp1 and a part of Nsp2 genes of a synthetic porcine reproductive and respiratory syndrome virus are responsible for the viral capacity to induce type I interferons

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Type I interferons (IFNs) play a critical role both in innate resistance to viral infection and in regulating the host adaptive immune response. Porcine reproductive and respiratory syndrome virus (PRRSV) is well characterized for its ability to suppress the production of type I IFNs. We recently generated a synthetic PRRSV strain whose genome was rationally designed based on a set of 59 full genome sequences of type II PRRSV. This synthetic PRRSV-CON virus confers outstanding levels of heterologous protection as compared with the prototype PRRSV strain FL12. Unlike most of naturally occurring PRRSV strains, the PRRSV-CON virus induces robust levels of type I IFN response in vitro, rather than suppressing such response. The ultimate objective of this study is to identify which genes (or parts thereof) of this synthetic PRRSV-CON virus are responsible for inducing type-I IFNs. Through the use of both gain and loss of function studies, we found that the 5' end fragment of PRRSV-CON genome encoding non-structural protein (nsp) 1-alpha, 1-beta and a part of nsp2 genome is solely responsible for inducing type-I IFNs. We are in the process of further mapping individual viral genes involved in induction of type-I IFNs. Collectively, the results obtained from this study may be beneficial for the rational development of more effective PRRS vaccine.

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Nanoparticle based Vaccination strategy against Swine Influenza Virus

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Pigs are believed to be one of the important sources of emerging human and swine influenza viruses (SwIV). Earlier we have shown that biodegradable polymer, PLGA (poly lactic-co-glycolic acid) nanoparticles (NPs), based porcine reproductive and respiratory syndrome virus vaccine administered intranasally to pig induces heterologous protection. NPs based swine influenza A virus (IAV) vaccine formulation and its intranasal delivery may be a potential vaccination strategy in pigs. In a recently completed study, H1N1 IAV conserved peptides cocktail vaccine

induced epitope specific T cell response with substantial clearance of heterologous virus from the lungs of pigs. In this study, PLGA NPs-entrapped inactivated H1N2 SwIV candidate vaccine was developed, and evaluated it's breadth of immunity against a H1N1 virus challenge in pigs. Our results in pre-challenged pigs at day post-vaccination 35 indicated enhanced antigen specific lymphocyte proliferation, and increased frequency of NK cell, γδ T cell, CD8+ and CD4 CD8 double positive T cells in PLGA NPs vaccine inoculated animals. Clinically, until 6 days post-challenge the PLGA NPs based SwIV vaccine received pigs had no clinical flu symptoms, supported with substantially reduced nasal virus shedding and significantly reduced gross lung lesions; and detectable infectious challenged SwIV was observed in the BAL fluid of only 20% pigs. Hemagglutination inhibition titers against the H1N2 virus was relatively high in PLGA NPs vaccine received compared to other pig groups. Overall, our results suggested moderate levels of cross-protective response in PLGA NPs H1N2 SwIV vaccinated pigs. This project was supported by USDA-AFRI, Nanovaccine Research Initiative at Iowa State University, and OARDC, The Ohio State University.

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The impact of abomasal infusion of linoleic acid or linolenic acid on plasma fatty acid and oxylipid biosynthesis following *Streptococcus uberis* exposure

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The bovine mammary gland is especially vulnerable to bacterial infection potentially resulting in severe inflammatory responses. *Streptococcus uberis* causes severe mammary tissue damage due to uncontrolled inflammation. The balance of 2 polyunsaturated fatty acids (PUFA), linoleic acid (C18:2 n-6, LA) and linolenic acid (C18:3 n-3, LNA), and their oxylipid derivatives can influence the inflammatory response. The LA-derived oxylipids are largely pro-inflammatory, whereas LNA-derived oxylipids are generally anti-inflammatory. Thus, our hypothesis is that white blood cells isolated from cows infused with LNA will have a reduced pro-inflammatory response to *S. uberis* compared to LA-infusioned cows. To address the hypothesis, we used 5 cows in a balanced Latin-square cross-over design and blood samples were taken before and after no abomasal infusion, 7 d LA abomasal infusion (45 g/d), or 7 d LNA abomasal infusion (45 g/d). Whole blood was then *ex vivo* stimulated with heat-killed *S. uberis* and liquid chromatography-tandem mass spectrometry was used to quantify 57 metabolites, including plasma fatty acids and oxylipids. There was no significant change in oxylipid biosynthesis following abomasal infusion in the absence of *S. uberis*. In contrast, one metabolic pathway for oxylipid biosynthesis, cytochrome P450 (CYP), was altered significantly in response to *S. uberis* stimulation. The CYP enzyme is an epoxygenase that oxidizes PUFA into epoxy oxylipids, which are then hydrolyzed to dihydroxy oxylipids. The LA infusion resulted in significantly less CYP-derived, 12,13-epoxyoctadecenoic acid (12,13-EpOME), 14,15-dihydroxyeicosatetraenoic acid (14,15-diHETE), and 14,15-dihydroxyeicosatrienoic acid (14,15-dHET) following *S. uberis* challenge. Research suggests some CYP-derived oxylipids may have an anti-inflammatory function, however, several studies highlight a possible role for dihydroxy oxylipids in eliciting leukocyte chemotaxis. The CYP pathway is not well described, and additional research is necessary to understand how changing CYP oxidation and biosynthesis of epoxy and dihydroxy oxylipids modifies the inflammatory response during mastitis.

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Bovine gamma delta T cells and Th17 cells produce IL-17 in response to respiratory syncytial virus and Mannheimia haemolytica: implications for bovine respiratory disease

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Bovine respiratory disease (BRD) results in substantial economic losses to both the beef and dairy industries. Bovine respiratory syncytial virus (BRSV) is a significant cause of morbidity and mortality in uncomplicated viral infections in the calf, and a principal factor in the development of BRD. Mannheimia haemolytica is a predominant bacterial isolate recovered from cases of bovine pneumonia and a leading cause of direct economic loss from BRD in the United States. During BRD, primary viral infection by pathogens such as BRSV significantly predisposes the calf to the development of bacterial pneumonia, caused by pathogens such as M. haemolytica. Currently, we understand little about the immune mechanisms and host pathogen interactions underlying the increased disease susceptibility observed in calves with BRD. Interleukin-17A (IL-17) is a pro-inflammatory cytokine that plays a critical role in the immune response in sites such as the respiratory tract and mucosa. While protective in a number of cases, IL-17 can also lead to damaging immunopathology, particularly through its role as a potent inducer of neutrophil recruitment and activation. Given its known importance in protection, and possibly immunopathology in the lungs during respiratory infection, we hypothesized that IL-17 may be playing a role in the immune response to BRD in the calf. We demonstrate here for the first time that IL-17 is expressed at high levels in the lungs of calves with severe BRSV infection and in calves with M. haemolytica infection. In recall responses, CD4 and gamma delta T cells from vaccinated or BRSV infected calves secrete significant concentrations of IL-17 protein and express high levels of the IL-17 associated cytokines IL-21 and IL-22. In vitro infection of PBMC or purified gamma delta T cell cultures with BRSV or M. haemolytica results in increased production of IL-17; and this response is exacerbated in cultures that are co-infected with both pathogens. Together, our results suggest that IL-17 is likely contributing to the immune response to BRSV and M. haemolytica infection in the calf; and that exacerbated IL-17 production may be a contributing factor to the exacerbated disease observed during BRD.

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First encounters: Mucosal immune system development and the microbiome

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Purpose:The mucosal immune system of the newborn calf is faced with a challenging transition from the sterile environment of the uterus to rapid colonization of the gastro-intestinal tract by a diverse community of commensal bacteria. This colonization begins during birth and is followed by a rapid change in both bacterial density and diversity during the first few days of life. Understanding how this early host-microbiome interaction

influences mucosal immune system development is critical for developing more effective strategies, including vaccination, to reduce the risk of enteric infection.
Methods: Evidence is now emerging that during the neonatal period there is a co-evolution of host immunity and the microbiome. This co-evolution is reflected in regional differences in both mucosal immunity and microbial communities throughout the gastro-intestinal tract. Presently, little is known about the mechanisms by which this first encounter shapes development of the mucosal immune system and the epithelial barrier. Programmed developmental events, colostrum, and the gut microbiome may interact in a variety of ways to alter mucosal immune system development.
Results: Transcriptomic analysis of both mRNA and micro(mi)RNA confirms that the first week of life is a very dynamic developmental period in the bovine small intestine. Furthermore, bacterial density was significantly correlated with differential expression of miRNAs implicated in immune cell development. It is much more difficult, however, to determine if bacterial diversity is also important or if individual bacterial species play key roles as part of a species-specific microbiome.
Conclusions:< The challenge remains to develop model systems to analyze regional interactions between host and microbiome throughout the gastro-intestinal tract of the newborn calf. It will then be important to determine if perturbations in these early encounters result in long-term effects on health and productivity.

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Mucosal immunology: microbial interaction and cytokine production

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Mucosal immune responses involve the immune modulation of the mucosa epithelial cells. These cells respond to microbial produced ligands and metabolites and modulate the immune response. This talk will review the current knowledge of the immune response of the mucosa, its interaction with the underlying cells of the immune system in the lamina propria and the role of microbial produced ligands and metabolites. An experimental approach in cattle to study these interactions will be discussed along with the proof of concept immunological measurements. Studying specific localized responses will require novel approaches.

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An essential role of igt for pathogen clearance and microbiome homeostasis at mucosal surfaces of fish

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Most fish pathogens enter their host through mucosal surfaces. The skin, gut and gill represent the largest mucosal surfaces in fish and they all contain a mucosal-associated lymphoid tissue (MALT). We have previously demonstrated that pathogen-specific mucosal antibody responses in the gut and skin are overwhelmingly mediated by the IgT antibody class. More recently we have also shown the IgT is the main player in gill immune responses. In addition, we have shown that IgT is the main Ig isotype coating commensal bacteria and thus, it plays a key function in immune exclusion. While these IgT activities point to a pivotal role of this immunoglobulin in teleost mucosal immunity, whether IgT is required for pathogen clearance and commensal homeostasis remains to be demonstrated. To address this critical question we have developed a unique IgT^+ B-cell depletion trout model. Upon depletion treatment, IgT^+ B- cells from all mucosal and systemic sites analyzed were depleted by over 95% for a 7 week period. In contrast the % of IgM^+ B cells did not change. Upon IgT^+ B-cell depletion, fish where sublethally challenged with *Ichthyophthirius multifiliis* or *Flavobacterium columnare*. After two weeks post-challenge, a significant percentage of mortality occurred in the IgT^+ B-cell depleted groups (25-50%). Critically, pathogen load was dramatically higher in the IgT^+ B-cell depleted groups when compared to control fish. In addition, IgT coating of commensals had for the most part disappeared 3 weeks post-depletion treatment and the microbiome at those mucosal surfaces was significantly changed. Interestingly we could never observe IgM or IgD compensatory responses against the pathogens or commensals. In conclusion we demonstrate that IgT is essential for pathogen clearance at mucosal surfaces and it plays a critical role in the maintenance of microbiota homeostasis. This represents the first non-mammalian model in which a specialized mucosal immunoglobulin and the B cells producing it are depleted. Importantly, this novel IgT^+ B-cell depletion model will be critical to understand further the role of IgT in host-pathogen interactions at mucosal surfaces.

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Inflammatory mediator expression in lung epithelial cells and α/β T cells: roles in immunopathogenesis associated with respiratory syncytial virus infection in calves

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Respiratory syncytial virus (RSV) is the most common viral cause of childhood acute lower respiratory tract infections. Bovine RSV is a cause of enzootic pneumonia in young dairy calves and summer pneumonia in nursing beef calves. Furthermore, bovine RSV plays a significant role in bovine respiratory disease complex, the most prevalent cause of morbidity and mortality in feedlot cattle. Infection of calves with bovine RSV shares features in common with RSV infection in children, including comparable microscopic lesions consisting of bronchiolar neutrophilic infiltrates, epithelial cell necrosis, and syncytial cell formation are observed. Recognition of viral PAMPs involves at least three distinct classes of pattern recognition receptors (PRRs), toll-like receptors (TLRs), retinoic acid inducible gene-I (RIG-I)-like receptors (RLRs), and nucleotide-binding oligomerization domain (NOD)-like receptors (NLRs). Ligation of cellular PRRs by viral PAMPs stimulates the release of inflammatory mediators. Our studies have shown an upregulation of pro-inflammatory chemokines in the lungs of RSV-infected calves, including CXCL8 (IL-8), CXCL10 (IP-10), CCL2 (MCP-1) and CCL3 (MIP-1α). These findings are consistent with the increased levels of these inflammatory mediators observed in children with RSV bronchiolitis and these chemotactic peptides have been implicated in RSV pathogenesis. Current

studies in the laboratory are focused on further characterization of inflammatory mediators in lung epithelial cells and α/β T cells in calves during BRSV infection.

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PIV-3 blocks antiviral mediators downstream of the IFN-λR by modulating Stat1 phosphorylation

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Type III interferon's (IFNs) signal through a unique heterodimeric receptor, the IFN-λR1/IL-10R2, which is primarily expressed by epithelial cells. Parainfluenza virus type 3 (PIV-3) infection is highly restricted to the airway epithelium. We therefore sought to examine type III IFN signaling pathways during PIV-3 infection. Paramyxoviruses are known to inhibit type I interferon (IFN) production, however there is a lack of information regarding the type III IFN response during infection. We used three strains of PIV-3: human PIV-3 (HPIV-3), bovine PIV-3 (BPIV-3), and dolphin PIV-1 (TtPIV-1). Here we show that message levels of IL-29 are significantly increased during PIV-3 infection, yet downstream antiviral signaling molecules are not upregulated to levels similar to the positive control. Furthermore, in Vero cells infected with PIV-3, stimulation with recombinant IL-29/28A/28B does not cause upregulation of downstream antiviral molecules, suggesting that PIV-3 interferes with the JAK/STAT pathway downstream of the IFN-λR1/IL-10R2 receptor. We used western blotting to examine the phosphorylation of Stat1 and Stat2 in Vero and BEAS-2B cells. In Vero cells we observed reduced phosphorylation of the serine 727 (S727) site on Stat1, while in BEAS-2B cells Stat1 was dephosphorylated at the tyrosine 701 (Y701) site during PIV-3 infection. PIV-3 therefore interferes with the phosphorylation of Stat1 downstream of the type III IFN receptor. It is important to examine PIV-3 infection of epithelial cells at the molecular level to aid in development of a successful vaccine.

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From Swine Dysentery to Inflammatory Bowel diseases: Role of the Resident Microbiota in Tuning the Host Response.

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Swine dysentery is a mucohemorrhagic diarrheal disease of pigs and the causative agent of clinical disease is *Brachyspira hyodysenteriae.* While *B. hyodysenteriae* acts as the match that lights the fire (i.e., mucosal inflammation), the pathogenesis of the disease requires the presence of a resident microbiota and the immune response of the host. Building upon these studies, we have more recently evaluated the interactions between the resident microbiota of the colon and hosts susceptibility to colitic insults. In this regard, we have used gnotobiotic mice colonized with the eight members of the altered Schaedler's flora. In order to perturb mucosal homeostasis, these gnotobiotic mice were colonized with either *Escherichia coli* or *Helicobacter bilis.* The impact of these bacterial provocateurs on mucosal homeostasis, adaptive immune responses, and increased sensitivity to DSS will be discussed.

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PEDV shedding patterns and antibody kinetics in commercial growing pigs

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Purpose:

Longitudinal samples collected from two production sites (one PEDV positive; one PEDV negative) were used to 1) describe the pattern of PEDV shedding (RT-PCR) in individual pig fecal swabs, pen fecal samples, and pen oral fluids (OF); 2) describe the kinetics of PEDV antibody by ELISA (IgA, IgG) testing of pig serum and pen OF samples; and 3) establish cutoffs and performance estimates for PEDV "whole virus" IgA and IgG ELISAs (PEDV WV ELISA).

Methods:

Farm 1 was a 52-pen WTF barn stocked with 800 pigs. Pen samples (feces and OFs) and pig samples (fecal swabs and serum) were collected from the same 6 pens and a convenience sample of 5 pigs in each of the 6 pens at placement and at 2-week intervals for 27 weeks. At 13 weeks of age, this PEDV-negative population was exposed to PEDV using standard field exposure methods.

Farm 2 consisted of 3 identical 40-pen WTF barns, each stocked with 900 pigs. Pen OF samples were collected from 36 pens in each of the 3 barns and serum samples were collected from a convenience sample of 20 pigs in 2 pens (10 pigs per pen) in each barn. Sampling began at placement and was done weekly for a total of 9 samplings.

Pen feces, OFs and fecal swabs were tested by PEDV RT-PCR; OF and sera were tested by PEDV WV ELISA (IgG, IgA) at the ISU VDL.

Results:

On Farm 1, PEDV was detected by RT-PCR at the first sampling post inoculation (DPI 6) in individual fecal swabs, pen fecal samples and pen OF. The last RT-PCR positives were detected in fecal swabs and OFs on 69 DPI. Overall, the highest percent of positive samples was observed in OF. Anti-PEDV IgG and IgA was detected in OF and serum samples collected at 13 DPI. The OF IgA response increased through 97 DPI, while serum IgA responses peaked at 27 DPI.

Farm 2 remained RT-PCR negative throughout the monitoring period. These samples provide a source of negative samples for calculating cutoffs and performance estimates for the WV IgA and IgG ELISAs (analysis in progress).

Conclusions:

PEDV antibody (IgG and IgA) kinetics in OFs and comparison with the serum antibody responses has not previously been reported. Future work will focus on establishing antibody levels associated with cessation of PEDV shedding and/or protection against infection.

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Cellular response following Digital Dermatitis infection

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Introduction: Digital dermatitis is an infectious cause of lameness, primarily affecting dairy cattle, but also beef cattle, sheep, goats, and a small population of North American wild elk (Cervus elaphus). Several *Treponema* spp. have been isolated from digital dermatitis lesions, and there is a strong association with other anaerobic bacteria. Data based on serum antibody responses indicate that immunity to digital dermatitis is short lived. Few investigations have been made into the cellular response elicited by infection.

Materials& Methods: Peripheral blood mononuclear cells (PBMCs) were isolated from cattleor wild elk which were known to be free of digital dermatitis, or which had digital dermatitis within the past 6 months. Diagnosis was made by visual inspection during routine hoof trimming or at the time of blood collection. PBMCs were stimulated with bacteria derived from digital dermatitis lesions. PBMCs were analyzed for proliferation and phenotype by flow cytometry.

Results: PBMCs from animals with digital dermatitis proliferated to treponemal antigens in a dose dependent manner after 5 days of simulation. The majority of the PBMCs proliferating were B cells, which supports the strong serum antibody response observed in these animals. In bovines, there was a significant population of proliferating γδ-TCR+ cells; however, in both bovine and elk PBMCs, there were a significant number CD4+ and CD8+ cells proliferating in response to stimulation with treponemal antigen.

Discussion/Conclusion: In order to develop effective vaccines or other targeted interventions, it is necessary to understand the natural disease process, including the cellular immune response to bacteria key in development of the disease. These results suggest that during or shortly after active infection, there is specific acquired immunity as indicated by the antigen specific proliferation of CD4+ and CD8+ PBMCs. Further investigation is needed to determine the length of immunity, memory phenotype and other characteristics of these antigen specific cellular responses.

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Montanide™ adjuvant technologies for influenza vaccines.

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Influenza viruses infect diverse veterinary species, have a strong impact on meat production, and are also a concern for human health. Vaccination is the most effective way to control influenza in the field. Classical influenza vaccines used in the field are generally based on inactivated whole or modified virus antigens, which require the addition of strong adjuvants to induce long term protection. Here we show that Montanide™ adjuvants allow the formulation of highly efficient inactivated swine influenza vaccines, and are also compatible with an Aujesky's disease live vaccine.

Inactivated vaccines against Swine influenza virus (SIV) were formulated with polymer adjuvant Montanide™ Gel 01 (Gel), with oil in water emulsion adjuvant Montanide™ ISA 15A VG (ISA 15A) or without adjuvant. At day 0 and day 21, 10 seronegative pigs were vaccinated in each group simultaneously with 2ml of the corresponding inactivated SIV vaccine and 2ml of Aujesky's disease attenuated live vaccine. Safety properties of the vaccines were assessed at vaccination, during the trial and at slaughter. Efficacy was assessed both by serological analysis and application of infective challenge procedure against SIV and Aujesky's disease.

All vaccines were safe. Antibody titers against SIV were significantly superior for Gel or ISA 15A adjuvanted formulations compared to the non adjuvanted vaccine. Protection against SIV challenge was also improved, as hyperthermia after infection, lung lesions at slaughter and nasal viral shedding were reduced compared both to non vaccinated and non adjuvanted groups. Both adjuvants were compatible with Aujesky's disease live vaccine. Moreover, viral shedding after Aujesky's challenge was reduced in the Gel adjuvanted group.

These results show that relevant Montanide™ aqueous adjuvants can be compatible with both inactivated and attenuated viral vaccines for swine, are highly effective in improving SIV vaccine protective efficacy and do not impair the efficacy of co-administered live vaccine. Such adjuvants can allow the formulation of multivalent combined inactivated/live vaccines, which could lead to the reduction of the number of injections given to pigs in the field.

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Expression of interferon-beta (IFN-β) by dendritic cells activated with Streptococcus suis

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Streptococcus suis serotype 2 is an important porcine bacterial pathogen and emerging zoonotic agent responsible for sepsis and meningitis. North American (NA) strains present lower virulence compared to their European (EU) counterparts, while a Chinese (CH) strain responsible for two human outbreaks presents an even higher virulence. Although IFN-β production is usually associated with viral infections, it has also been reported during infections by extracellular bacteria. It was previously shown that a NA strain induces higher IFN-β levels than the EU and CH strains during the acute systemic infection in a mouse model of infection. Since the source of this production remains unknown, the expression of IFN-β by murine bone marrow-derived dendritic cells (bmDCs) activated by different strains of *S. suis* was evaluated. Cells were challenged with

NA, EU or CH *S. suis* serotype 2 strains and the IFN-β expression and production evaluated by RT-qPCR and ELISA, respectively. Results demonstrated that *S. suis* serotype 2-infected bmDCs are an important source of IFN-β, whose expression peaked at 6 hours. Moreover, IFN-β was significantly more induced by the NA strain, whereas levels were alike between the virulent EU and highly virulent CH strain-infected bmDCs. *S. suis* serotype 2 also induced the interferon regulatory factors 1 and 7 in bmDCs. Using well-characterized *S. suis* serotype 2 mutants, an important role of cell wall components was demonstrated since a non-encapsulated mutant induced significantly higher levels of IFN-β than its parental strain. Similarly, the suilysin (SLY), a cholesterol-dependent cytolysin, contributed to IFN-β expression. However, the NA strain, which does not produce SLY, induced higher IFN-β levels than the SLY-positive EU/CH strains, suggesting the contribution of different factors to the regulation of type I IFNs. Thus, these results confirm that a low-virulence *S. suis* strain induces high levels of IFN-β, suggesting a protective effect of IFN-β as described for other pathogenic streptococci. The contribution of macrophages to the IFN-β production as well as the confirmation of these observations with swine cells are presently under evaluation.

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Intramammary 25-hydroxyvitamin D3 treatment increases vitamin D pathway activity but not acute host-defense responses to endotoxin-induced mastitis.

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Purpose: The macrophage vitamin D pathway is activated in the mammary gland during mastitis and 25-hydroxyvitamin D3 (25D), the substrate for the macrophage vitamin D pathway, has been shown to decrease severity of mastitis in dairy cattle. Treatment of monocyte cultures with 25D increases monocyte iNOS and β-defensin expression, so it was hypothesized that intramammary 25D treatment also would enhance iNOS and β-defensin expression during mastitis in dairy cattle. The objective of this study was to determine the effects of intramammary 25D on mammary host-defense genes in endotoxin-induced mastitis.

Methods: Each mammary quarter of five lactating Holstein cows were treated with either 5 μg of lipopolysaccharide (LPS), 100 μg of 25D, a combination of 100 μg 25D and 5 μg LPS (LPS+25D), or placebo (10 mL of phosphate-buffered saline with 10% fetal bovine serum). Somatic cells were collected at 0, 4, 8, 12, 24, 36, 48, and 72 h relative to the challenge and evaluated for expression of vitamin D pathway, β-defensin and iNOS genes.

Results: The 1α-hydroxylase (CYP27B1, 1,25D synthesis), 24-hydroxylase gene (CYP24A1, upregulated by 1,25D and responsible for vitamin D catabolism) and vitamin D receptor genes were upregulated in response to LPS in neutrophil and macrophage populations by 8 h post-challenge ($P < 0.01$). The β-defensin genes (BD3, BD4, BD7, and BD10) and iNOS were upregulated in response to LPS in total somatic cell and neutrophil populations. The 25D treatment alone and in combination with LPS upregulated CYP24A1 expression ($P < 0.05$) but did not have an effect on β-defensin or iNOS gene expression.

Conclusions: Endotoxin challenge rapidly activates the vitamin D pathway in the mammary gland but intramammary 25D treatment does not affect the acute induction of β-defensin and iNOS gene expression in the mammary gland.

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A novel vector platform for vaccine delivery in domestic animal species

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The parapoxvirus ORF virus (ORFV) presents several unique properties that make the virus an excellent candidate for vaccine delivery in livestock species. Notably, the virus encodes a complement of immunomodulatory proteins that target host immune responses against infection. The goal of this study was to identify an ORFV-based vector that is safe and immunogenic in domestic animal species (cattle, pigs and horses). For this, we constructed ORFV recombinants expressing a model viral antigen, the rabies virus (RABV) glycoprotein G (gG), and evaluated their immunogenicity in target animal species. Expression of gG by recombinants ORFV-RabVgG-1 and ORFV-RabVgG-2 was confirmed in vitro and replication characteristics of the recombinant viruses were assessed in cell cultures derived from target animal species. The immunogenicity of ORFV-RabVgG-1 and ORFV-RabVgG-2 recombinant viruses was assessed in cattle pigs and horses. Animals from each species were divided in two groups and immunized intramuscularly with two doses (21 days interval) of ORFV-RabVgG-1 or ORFV-RabVgG-2 containing 107.9 TCID50/dose. No adverse reactions were observed after immunization with the recombinant ORFV vectors. Levels of neutralizing antibodies against rabies virus were assessed on days 0 (vaccination day), 21 (second dose) and 42 (21 days after re-vaccination) by rapid fluorescent focus inhibition test (RFFIT). All immunized animals developed VN antibodies to RabV by day 21 post-revaccination. Notably, immunization with ORFV-RabVgG-2 elicited higher levels of RabV neutralizing antibodies when compared to those induced by ORFV-RabVgG-1-immunization. These results demonstrate the suitability of ORFV as a vaccine delivery vector in cattle, pigs and horses.

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Porcine reproductive and respiratory syndrome virus non-structural protein Nsp2TF down-modulates Swine Leukocyte Antigen class I (SLA class I) expression

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Porcine reproductive and respiratory syndrome virus (PRRSV) is an economically important swine pathogen which typically induces sub-optimal immune responses thereby leading to a persistent infection. The sub-optimal immune responses against PRRSV are partly due to a delayed induction and impaired effector function of the cell-mediated immunity (CMI). Previous studies showed that PRRSV reduced the surface expression of swine leukocyte antigen class I (SLA-I) in susceptible host cells such as macrophages and dendritic cells, which may be partly

responsible for the impaired CMI responses against PRRSV. In this study, we first verified the previous observations in a porcine kidney epithelial stable cell line expressing CD163 (PK15-CD163) infected with a PRRSV strain VR2385. Subsequently we investigated the PRRSV proteins responsible for SLA-I modulation and demonstrated that the viral proteins Nsp1a, Nsp2TF and GP3 significantly down-regulated the surface expression of SLA-I, with Nsp2TF showing the greatest potential of reduction. To further confirm whether Nsp2TF reduces SLA-I expression in the context of viral infection, a mutant virus (PRRSVΔNsp2TF) was generated using a DNA-launched PRRSV infectious clone in which the frameshifting elements that produced Nsp2TF were completely disrupted to knock out its expression. Additionally, to reinforce the knockout, several mutant viruses were generated with the introduction of additional stop codons in the TF domain of the protein. The one with 3 additional stop codons (PRRSVΔNsp2TF-3UAGs) reversed SLA-I surface expression when compared to the wild-type virus infection. The results indicate that Nsp2TF down-regulate SLA-I expression during PRRSV infection. The results from this study identified a novel function for Nsp2TF in the negative modulation of SLA-I expression. Since antigen presentation associated with SLA-I molecules is essential for the activation of or recognition by T cells, the Nsp2TF may directly play a role in PRRSV-mediated immune modulation. Therefore, disrupting the Nsp2TF's ability to affect the SLA-I expression may improve the existing PRRSV vaccines towards a better CMI response against the virus.

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Endonuclease G participates in caspase-independent apoptosis induced by Mycobacterium bovis in bovine macrophages.

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Mycobacterium bovis the causative agent of bovine tuberculosis brings into play different virulence factors to survive inside of host cells. One of the possible outcomes in this scenario is cell death. Our research group reported for the first time bovine macrophage apoptosis induction associated to Mycobacterium bovis infection. In addition, we demonstrated macrophage Apoptosis Inducing Factor (AIF) nuclear translocation as a consequence of infection. However, the role of other macrophage proteins in this process is not well understood. In this study, we aimed to identify Endonuclease G (Endo G) involvement in M. bovis apoptosis induction. Nuclear protein extracts from M. bovis- infected and uninfected cells were analyzed by immunoblot. Endo G nuclear concentration in infected cells was around 2-fold when compared to uninfected macrophages. We used Cyclosporine A (CsA) to inhibit macrophage mitochondrial permeability transition and measure nuclear AIF and Endo G by immunoblot. CsA treatment of infected macrophages abolished nuclear translocation of AIF and Endo G. Furthermore, the number of TUNEL positive cells under these experimental conditions was very similar to non-infected cells (0.8 fold). Our results suggest that Endo G plays an important role in M. bovis-infected macrophages apoptosis signaling pathway. This work was supported by project PAPIIT IN-217512-2 and CONACYT CB-167488. A. Benitez-Guzmán received a postdoctoral fellowship from DGAPA, UNAM.

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Pathogenesis comparison of the U.S. PEDV prototype and S-INDEL-variant strains in weaned pigs and examination of the cross-protective immunity of two virus strains

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At least two genetically different porcine epidemic diarrhea virus (PEDV) strains are circulating in U.S. swine: U.S. PEDV prototype and S-INDEL-variant strains. The objectives of this study were to evaluate the pathogenesis differences and cross-protection efficacy of the two strains in weaned pigs.

Eighty-five PEDV-naïve 3-week-old pigs were divided into 7 groups. Pigs were orogastrically inoculated with negative culture media (N), a PEDV prototype isolate (P), or an S-INDEL-variant isolate (V) at Day 0 (D0) followed by challenge at D28, using inoculum of 105 TCID50/pig at each point. Seven groups were designated according to 1st inoculation/2nd challenge: P/V (15 pigs), V/V (15 pigs), N/V (15 pigs), P/P (10 pigs), V/P (10 pigs), N/P (10 pigs), N/N (10 pigs). Five pigs from the P/V, V/V, and N/V groups were necropsied at D4 and 5 pigs from all 7 groups were necropsied 6 days after the 2nd challenge (D34) to evaluate gross and microscopic lesions. The remaining 5 pigs per group were kept until D56 to evaluate post-challenge antibody response.

The P/V and P/P groups shed higher amount of virus in feces than the V/P and V/V groups during D0-7. Interestingly, after the 2nd challenge at D28 (pigs 7 weeks old), the N/V group shed more viruses in feces than the N/P group during D32-38. All pigs in the P/P, P/V, V/V and V/P groups developed PEDV IFA and neutralizing antibodies starting from D7-14; antibody titers increased slightly after the 2nd challenge and were maintained through D56. The N/P and N/V groups were PEDV antibody negative until D42 when antibodies became detectable and were maintained through D56. The N/N group remained virus and antibody negative from D0-56. After the 2nd challenge, fecal viral shedding in the P/P, V/P, V/V, and P/V groups were all significantly less than detected in the N/V and N/P groups. Evaluation of histopathologic lesions and immunohistochemistry staining are in progress.

In summary, the U.S. PEDV prototype strain appeared to achieve higher levels of fecal virus shedding than S-INDEL-variant strain in 3-week-old pigs but the opposite was observed in 7-week-old pigs. Both U.S. PEDV strains provided homologous and heterologous protection against two strains in weaned pigs.

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Genome wide association study identifies loci associated with somatic cell count phenotypes following experimental challenge with Streptococcus uberis

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Mastitis is a detrimental disease in the dairy industry that costs upwards of $2 billion annually and decreases milk quality. Often, mastitis results from bacteria entering the gland through the teat end. A common mastitis causing pathogen is *S. uberis*, which is responsible for 14-26% of subclinical and clinical mastitis cases. Following an intramammary experimental challenge with *S. uberis* on Holstein cows (n=40), milk samples were collected and somatic cell counts (SCC) were determined by the Dairy Herd Improvement Association Laboratory. Traditional GWAS have utilized SCC (cells/ml) over the course of lactation to identify loci of interest for mastitis. Our approach uses SCC during an experimental intramammary challenge to generate three novel phenotypes: area under the curve (AUC) of SCC for 0-7 d and 0-28 d post-challenge; and time to return to SCC <200,000 cells/ml (<21 d, 21-28 d, or >28 d). To identify loci of interest a 50K SNP chip analysis was performed using the BovineSNP50 v2 DNA Analysis BeadChip from Illumina. Associations were tested using Plink. Preliminary analyses revealed 16 highly significant ($p<1.0x10^{-5}$) SNPs across the 3 phenotypes. Of these, 4 are part of significant SNP clusters on BTAs 9 and 20 and the SNP in the BTA9 cluster is directly in an immune related gene. The identified loci should be further investigated to potentially identify causation behind the observed phenotypes. Such investigations could lead to novel treatment and prevention compounds/protocols for mastitis and/or genetic selection methods for cows with greater potential to resist or tolerate infection.

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Bordetella bronchiseptica colonization has minimal impact on live-attenuated influenza virus vaccine cross-protective efficacy in pigs.

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Influenza A virus (IAV) is a major animal and public health concern for the swine industry. The large number of IAV H1 and H3 variants co-circulating in US swine has made control of IAV very difficult. Live-attenuated influenza virus (LAIV) vaccines delivered by the intranasal route have been shown experimentally to provide protection against heterologous IAV of the same subtype. Immunogenicity in the respiratory tract is the primary mechanism by which LAIV vaccines provide significant cross-protection, limiting viral replication throughout the respiratory tract. However, it's possible that the respiratory tract microbial flora could impact LAIV immunogenicity and efficacy. Given the ubiquitous presence of *Bordetella bronchiseptica* in swine, it's ability to alter host immune responses, and the importance LAIV immunogenicity in the respiratory tract, a study was performed to determine if *B. bronchiseptica* colonization prior to LAIV vaccination altered LAIV efficacy against heterologous IAV challenge. Overall, *B. bronchiseptica* colonization prior to LAIV administration did have an impact on LAIV cross-protective efficacy, though the impact was limited. Specifically, titers of IAV in the trachea on day 5 post-infection were greater in pigs vaccinated with LAIV in the presence of *B. bronchiseptica* when compared to pigs administered LAIV without *B. bronchiseptica*. Titers of IAV in nasal swabs were also increased, but only on days 1 and 2 post-infection. Pneumonia scores were higher in pigs colonized with *B. bronchiseptica* and challenged with IAV, regardless of LAIV vaccination status. Overall, these data show that LAIV vaccination provides cross-protection against heterologous IAV challenge and efficacy is minimally impacted by coincident *B. bronchiseptica* colonization.

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Characterization and application of monoclonal antibodies against porcine epidemic diarrhea virus

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Purpose: Porcine epidemic diarrhea virus (PEDV) causes acute diarrhea to pigs at all ages, resulting in high mortality in piglets less than one week old. Since April 2013, PEDV has rapidly spread in the US and causes the loss of over 10% of the US pig population.

Methods: Monoclonal antibody (mAb) is a key reagent for rapid diagnosis of PEDV infection. In this study, we produced a panel of mAbs against nonstructural protein 8 (nsp8), spike(S) protein, and nucleocapsid (N) protein of PEDV. Three mAbs were selected, which can be used in various diagnostic assays, including immunofluorescence assay, enzyme-linked immunoabsorbent assay (ELISA), Western Blot, immunoprecipitation (IP), immunohistochemistry (IHC) test and fluorescence in situ hybridization (FISH).

Results: The mAb 51-79 recognizes amino acid (aa) 33-60 of nsp8, and mAb 70-100 recognizes aa1371-1377 of S2 protein, while mAb 32-20 recognizes aa47-93 of N protein. Using the mAb70-100, the immunoprecipitated S2 fragment was examined by protein N-terminal sequencing, and cleavage site between S1 and S2 was identified. In addition, this panel of mAbs was further applied to determine the infection site of PEDV in the pig intestine. IHC test result showed that PEDV mainly located at the mid jejunum, distal jejunum and ileum.

Conclusions: Results from this study demonstrated that this panel of mAbs provides a useful tool for PEDV diagnostics and pathogenesis studies.

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Pathogenicity and physicochemical properties of *Salmonella* Typhimurium treated with natural phenolics from industry byproducts

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Purpose: The purpose of this study was to investigate the phenotypic and genotypic alterations of *Salmonella* Typhimurium exposed to both lethal and sub-lethal concentrations of berry pomace phenolic extracts.

Methods: Bacterial growth pattern, cell surface hydrophobicity, auto-aggregation capability were determined. Adhesion and invasiveness assay was carried out in cell-culture model. Expression of virulence genes was determined with qRT-PCR.

Results: Minimum bactericidal concentration of blackberry and blueberry pomace extracts (BPE) were 1.5 and 1.8 mg GAE/mL on *S.* Typhimurium. Treatment with sub-lethal concentrations of berry pomace extracts decreased the cell surface hydrophobicity and auto-aggregation capacity by ~50% and significantly increased the percentage of injured cells. Interaction of *S.* Typhimurium with cultured host cells were altered significantly; BPE reduced bacterial invasion into human intestinal epithelial cells (INT407) by 3 logs, in chicken fibroblast cells (DF1) by >2 logs and in chicken macrophage cells (HD11) by ~ 1 log, compared to the control. The relative expression of *hilA*, *hilC*, *invA*, *invE*, *invF*, *sirA*, and *sirB* genes in *S.* Typhimurium were down-regulated whereas *invC* was upregulated, due to treatment with BPE. In chicken model, 0.5 and 1.0 mg GAE/mL BPE as water supplement reduced the natural colonization of *Salmonella* in chicken cecum by > 2 logs compared to the control

group.
Conclusions: This study shows that bioactive extracts from berry pomace can serve as a potential alternative to synthetic antimicrobials and reduce *Salmonella* colonization in farm animals specifically poultry, to improve product safety.

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Enhancing food safety for human consumers by eliminating food-borne enteric pathogens

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Salmonella, Campylobacter and extra intestinal *Escherichia coli* pathovars (ExPEC, many of which are APEC) are leading food-borne pathogens worldwide and represent major human public health problems. Poultry and their eggs are a major source for transmission of these pathogens through the food chain to humans, although other farm animals, vegetables and fruits have also been implicated. Since *Salmonella* and *Campylobacter* are benign commensals in poultry, their elimination by development of vaccines affords no economic benefit to producers but rather an added expense. On the other hand, infections of poultry with *Clostridium perfringens* causing necrotic necrosis, *Eimeria* species causing coccidiosis and APEC infections causing colisepticemia (while also contributing to carcass condemnation at slaughter), do contribute economic losses in poultry production. We recently developed multiple new innovative means to construct recombinant attenuated *Salmonella* vaccine (RASV) strains with high immunogenicity, complete safety and attenuation, and inability to persist or be shed in an infective or viable form. We are now using these new technologies to develop RASVs that will prevent infections of poultry with *C. perfringens*, *Eimeria* species and APEC strains. These vaccines will also enhance food safety by eliminating the ability of any *S. enterica* serotype to infect and colonize chickens. To achieve these objectives we have modified *S.* Typhimurium-derived RASVs to display regulated delayed attenuation in vivo, regulated delayed in vivo synthesis of protective antigens specified by codon-optimized DNA sequences and regulated delayed lysis in vivo. The lysis phenotype permits RASVs to deliver in lymphoid tissues a bolus of protective antigen(s) and also confers complete biological containment. These vaccines are grown under conditions that enable them to display after course spray/oral administration the capabilities of a wild-type strain to survive host defense stresses and efficiently colonize effector lymphoid tissues before manifesting attenuation to preclude disease symptoms and to synthesize protein antigens to induce protective immune responses.

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Salmonella Pathogenicity Island 13 contributes to pathogenesis in streptomycin pre-treated mice but not in day-old chickens.

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Purpose: Salmonella enterica serovar Enteritidis (S. Enteritidis) is a human and animal pathogen that causes gastroenteritis characterized by inflammatory diarrhea and occasionally an invasive systemic infection. Salmonella pathogenicity islands (SPIs) are horizontally acquired genomic segments known to contribute to Salmonella pathogenesis. The objective of the current study was to determine the contribution of SPI-13 to S. Enteritidis pathogenesis.
Methods: We deleted the entire SPI-13 (ΔSPI-13) from the genome of S. Enteritidis CDC_2010K_0968 strain isolated from a human patient during the 2010 egg-associated outbreak in the US. The kinetics of infection of the wild-type (WT) parent and the ΔSPI-13 were compared in orally challenged day-old chickens and streptomycin pre-treated mice. The degree of intestinal inflammation and the survival of mutant strain within the avian (HD11) and murine (RAW264.7) macrophages were also determined.
Results: The deletion of the SPI-13 resulted in significantly impaired infection kinetics of S. Enteritidis in streptomycin pre-treated mice which was characterized by significantly lower ($P < 0.05$) viable counts in the ceca, liver and spleen, impaired ability to induce intestinal inflammation and reduced survival within murine macrophages. Conversely, there were no significant differences in the infection kinetics of ΔSPI-13 in day-old chickens in any of the organs tested and the survival of ΔSPI-13 within chicken macrophages remained unaltered.
Conclusions: The results of this study show that SPI-13 contributes to the pathogenesis of S. Enteritidis in streptomycin pre-treated mice but not in day-old chickens and raises the possibility that SPI-13 may play a role in pathogenesis and the host adaptation/restriction of Salmonella serovars.

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Salmonella pathogenicity island 13 contributes to the metabolic fitness of Salmonella Enteritidis through glucuronic acid and tyramine metabolism.

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Purpose:
Salmonella Pathogenicity Island-13 (SPI-13) is a polymorphic genomic island found in most sequenced Salmonella genomes. We previously reported that SPI-13 contributes to pathogenesis of an avian host adapted Salmonella enterica serovar Gallinarum in day-old chickens. Recently, we demonstrated that SPI-13 contributes to pathogenesis of a non-host adapted serotype S. Enteritidis in mice. However, the underlying mechanism by which SPI-13 contributes to pathogenesis remains unknown. Bioinformatic analysis suggests that most of the SPI-13 genes encode proteins that are predicted to be involved in bacterial metabolism, raising the possibility that SPI-13 may contribute to pathogenesis indirectly via enhanced metabolic fitness. Our hypothesis is that SPI-13 contributes to metabolic fitness of S. Enteritidis. The objective of this study was to identify metabolic phenotypes associated with SPI-13.
Methods:
The respiratory activity (RA) of a S. Enteritidis SPI-13 mutant (ΔSPI-13) and a wild-type parent strain (WT) for 952 different metabolic phenotypes was compared by OmniLog phenotype microarray (Biolog, USA) technology. Two independent screening experiments were conducted to identify metabolic substrates for which the RA activity of ΔSPI-13 strain differed significantly ($P < 0.05$) when compared with the

WT parent strain.

Results:

The ΔSPI-13 mutant was defective in utilization of tyramine as a sole source of carbon and nitrogen and D-glucuronic acid as a sole carbon source. Follow-up independent growth studies confirmed these phenotypes and showed that the growth of ΔSPI-13 mutant was significantly impaired when compared with the WT parent strain in media supplemented with tyramine or D-glucuronic acid. Consequently, we constructed several knockout mutants lacking one or more genes within SPI-13 to identify specific genes or gene clusters that contribute to metabolism of tyramine and D-glucuronic acid.

Conclusions:

We demonstrate that genes within SPI-13 contribute to tyramine and D-glucuronic acid metabolism. The results of these studies will be presented and relevance of these metabolic substrates within the host environment will be discussed.

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Quantification of coliforms and *Escherichia coli on beef carcasses immediately before and after evisceration during slaughter.*

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The evisceration process during beef cattle slaughter could contribute to carcass contamination by transferring bacteria from the gastrointestinal tract or via manual human contact during the process. Coliforms in general, and *E. coli* more specifically, have been used as indicators of carcass contamination with foodborne pathogens. However, published data on the prevalence and concentrations of these bacteria on carcasses immediately before and following evisceration are limited. Therefore, our objective was to compare the pre- and post-evisceration prevalence and concentration of coliforms and *E. coli* on the ventral surface of carcasses during beef slaughter. Over four processing days in a commercial beef processing facility, a 3,716 cm^2 area on each of 100 total carcasses were sponge sampled pre- and post-evisceration, by randomly alternating sampling between left and right sides of the carcass. Serial dilutions of each sample were prepared in buffered peptone water and plated in duplicate on 3M Petrifilm *E. coli*/Coliform count plates. Petrifilms were incubated at 37° C for 24 hours and enumerated for total coliforms and *E. coli*. The probability of carcasses testing positive for coliforms and *E. coli*, and log transformed concentration data, were analyzed using generalized and general mixed models, respectively, with sampling day as a random effect. The percentage of pre-evisceration carcasses positive for coliforms (25%) and *E. coli* (18%) was relatively low; mean concentrations across all carcasses were $2.3x10^1$ and $6.7x10^0$ CFU/100 cm^2, respectively. Following evisceration, most carcass samples were positive for coliforms (96%) and *E. coli* (70%); mean concentrations were $2.4x10^3$ and $1.1x10^3$ CFU/100 cm^2, respectively. The probability of a carcass sample testing positive for *E. coli* or coliforms was greater (*P* values < 0.05) post-evisceration than pre-evisceration, yet mean concentrations did not differ. Additional data from multiple beef processors are required to more fully quantify the influence of the evisceration process upon carcass contamination; however these data indicate that the probability of carcass contamination is greater following evisceration.

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Quantification of microbial transfer from hides to carcasses in commercial beef slaughter operations

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Shiga toxin-producing *Escherichia coli* (STEC) are foodborne pathogens of public health importance, which are found in the gastrointestinal tract of cattle. Growth patterns of STECs can be monitored using indicator organisms (coliforms, *E. coli, Enterobacteriaceae* (EB), and Aerobic Plate Counts (APC)). The objective of this study was to obtain data regarding the contamination levels and quantitative transfer of fecal-origin contamination (coliforms, *E. coli*, EB, and APC) from animal hides to carcasses in commercial operations. Hide and carcass samples were collected from four large commercial processing plants in KS, NE, and TX, which were visited 3 times each, during June and July 2015. Hide-on, pre-intervention pre-wash (hide-off), pre-evisceration, post-evisceration, and final product (post-interventions) carcass surface samples were collected. Twenty samples were collected from each sampling station during each plant visit. Hide-on and pre-intervention samples were collected from the same carcass to determine carcass contamination from the hides (fecal-origin). Pre- and post-evisceration samples were obtained from the same carcass to determine carcass contamination from evisceration. Final product samples were independent of the other sampling sites. Samples were plated on 3M Petri films; *E. coli*/coliform count plates, EB count plates, and APC plates. Overall mean and median (range) of *E. coli* plate counts were 4.82, 4.44 (<0 - 6.05) log CFU/100 cm^2 for hide-on and 1.17, 0.41 (<0 - 2.56) log CFU/100 cm^2 for pre-intervention samples. Overall mean and median (range) of *E. coli* plate counts for pre-evisceration samples were 1.00, <0 (<0 - 2.58) log CFU/100 cm^2 and for post-evisceration samples; 0.92, <0 (<0 - 2.69) log CFU/100 cm^2. Overall mean and median (range) of *E. coli* plate counts for final product samples were -2.27, <0 (<0 - -0.19) log CFU/100 cm^2, respectively. These data along with coliforms, EB and APC results from this study will be further described. Information about their distributions and potential contamination transfer will be utilized in a quantitative microbial risk analysis to model the risk of human illnesses due STEC along the beef chain.

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Bayesian estimation of sensitivity and specificity of culture- and PCR-based methods for the detection of the six major non-O157 Escherichia coli serogroups in cattle feces

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Cattle are the principal reservoir for Shiga toxin-producing E. coli (STEC) which they shed in their feces. The United States Department of Agriculture Food Safety and Inspection Service have declared six non-O157 STEC (O26, O45, O103, O111, O121, and O145) as adulterants in raw, non-intact beef products. PCR-based methods_conventional (cPCR) and real-time quantitative (qPCR)_have been developed for the detection of non-O157 STEC in cattle feces. The aim of this study was to generate sensitivity (Se) and specificity (Sp) estimates for PCR- and

culture-based methods used for the detection of the six non-O157 serogroups in cattle feces, using Bayesian latent class models.
A total of 576 freshly voided pen floor fecal samples were collected from pens of finishing cattle in a commercial feedlot in the United States during summer 2013. Fecal samples, suspended in E. coli broth, were enriched and subjected to three detection methods: cPCR, qPCR, and culture (immunomagnetic separation with serogroup specific beads and plating on a modified Posse medium). Samples were considered serogroup positive if the recovered isolate or extracted DNA tested positive for an O gene of interest; neither Shiga-toxin or intimin genes were assessed. The models assume conditional dependence of the PCR methods and perfect specificity for culture.
The Se and Sp of the methods are presented as low (median: 0.0-40.0%), medium (40.1-70.0%), or high (>70.0%). A low number of positives detected precluded estimation of O111 test performance. Culture Se estimate was low for O26 and O121, medium for O45, and high for O103 and O145 serogroups. For cPCR, Se estimates were medium for O45, O103, and O145, but high for O26 and O121. Sp was high for all serogroups. For qPCR, the Se estimate was medium for O145, but high for O26, O45, O103, and O121 serogroups. Sp was medium for O45, O103, and O121, but high for O26 and O145.
PCR-based tests, especially qPCR, were the most sensitive for the detection of non-O157 in feces of naturally-shedding cattle. The qPCR was less specific compared to cPCR or culture method. These data provide important estimates of test performance for calculating true prevalence and adjusting for test error in risk modeling.

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Associations between diet, gut microbiome, and health in red-shanked doucs (Pygathrix nemaeus): a model for captive primate health

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Red-shanked doucs (Pygathrix nemaeus) and other colobines possess specialized gastrointestinal systems similar to ruminants. They utilize both fore- and hindgut fermentation to meet their energetic demands. Maintenance of captive populations has been largely unsuccessful. Improving captive conditions is hindered by critical gaps in our understanding of their natural diet and enteric microbial adaptations that facilitate the digestive process. We used the douc as a model to study the relationships between diet and microbial community activity within the gastrointestinal tract. Fecal samples from 7 wild, 18 semi-captive, and 9 captive red-shanked doucs were collected between 2012-2013 from Son Tra NR (Vietnam), a Vietnamese sanctuary, and 2 zoological institutions. We measured gut microbiome composition using 16S rRNA sequencing. PICRUSt software was used to predict microbial function. Feeding behaviors of wild doucs were surveyed using focal sampling. Foraged plant species were collected and analyzed for nutrient content. Dietary records, including nutritional data, were provided by the captive facilities. Statistical analyses were performed to identify correlations between diet, gut microbiome, and animal status (captive vs. semi-captive vs. wild). Analysis of similarity revealed that gut microbial communities grouped by animal status (ANOSIM $R = 0.92$; $p = 0.001$). A reduction in gut microbiome richness and diversity was observed in captive doucs. Specifically, wild doucs (4231.68 ± 584.37 OTUs) harbored the highest number of OTUs (greatest diversity), followed by the semi-captive doucs (2845.50 ± 494.98 OTUs), and captive doucs (2534.78 ± 376.19 OTUs). The Firmicutes to Bacteroidetes ratio, which is a measure of energy harvest capacity, was higher in the wild population (4.64 ± 0.94) than in the semi-captive (3.78 ± 1.14) and captive (1.71 ± 0.72) populations. We identified microbial biomarkers of douc nutritional health, including Bacteroides and Prevotella, which were far more abundant captive populations compared to the wild and semi-captive populations. We hypothesize that captivity causes doucs to shift to severe gut dysbiosis, thereby resulting in GI issues.

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Lactobacillus reuteri derived-histamine suppress interleukin-6 by inhibiting H1-receptor downstream signaling in germ-free mice

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Probiotics may beneficially affect the disease course of patients with chronic immune-mediated disorders like inflammatory bowel disease and colorectal cancer (CRC) via modulation of the host immune responses. A recent pangenomic study showed that human-derived clade II *L. reuteri* strains contained a complete chromosomal histidine decarboxylase (*hdc)* gene cluster (genes *hdcA*, *hdcB*, *hdcP*) and have the genetic capacity to convert histidine to histamine. In our lab we found *L. reuteri* 6475 (clade II) derived-histamine suppressed TNF production in human myeloid cells. In addition, we also showed administration of *L. reuteri* to HDC knock-out mice bearing inflammation-associated colon cancer (IaCC) showed suppression of inflammatory cytokines mainly IL-6, IL-22, IL-1α and TNF. Apart from suppression of inflammatory response, $CD11b^{+}Gr\text{-}1^{+}$ immature myeloid cell (IMCs) populations were reduced in spleen and bone marrow of *hdcA*$^{+}$ *L. reuteri* administered to HDC KO mice bearing IaCC compared to HDC KO IaCC mice with *hdcA*$^{-}$ *L. reuteri*. These changes correlated with reduced tumor numbers in the HDC KO mice. However, *L. reuteri* with mutant *hdcA gene* were unable to produce histamine and neither protected the HDC KO mice from IaCC. However, *L. reuteri* 6475 derived histamine and its ability to suppress inflammation and reduce IaCC are not well understood. So, we hypothesized that *L. reuteri* derived-histamine down-regulates histamine receptor 1(H_1R) and allows H_2R activation, thereby suppressing inflammation. We used Swiss-Webster WT and BALB/c WT germ-free mice mono-associated with *L. reuteri* wild-type or the *L. reuteri hdcA* mutant strain. The result showed significant diminution of IL-6 expression in the GF mice receiving *L. reuteri* WT and *hdcA* mutant strain. In addition, we found that both WT and mutant strain can produce Diacylglycerol kinase (DAGK). DAGK can inhibit DAG signaling downstream. Therefore, we speculate that IL-6 is suppressed due to DAG inhibition of H_1R downstream signaling and regulates the H_2R signaling by *L. reuteri* histamine.

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Host defense peptide-inducing compounds as alternatives to antibiotics

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Subtherapeutic use of antibiotics in livestock production for growth promotion and disease prevention has potential to drive up antimicrobial resistance. Although various forms of alternatives to antibiotics have been explored, none is able to match the efficacy of antibiotics. Host defense peptides (HDPs) constitute a diverse group of small molecules that are an important component of innate immunity with potent antimicrobial, immune regulatory, and barrier function-enhancing activities. We recently discovered several classes of natural and synthetic small-molecule compounds that are highly efficient in specific induction of endogenous HDPs in the intestinal tract. Supplementation of these HDP-inducing compounds enhances bacterial clearance and intestinal barrier integrity with a tendency to improve animal production efficiency as well. Unlike most immunomodulators on the market, these HDP-inducing compounds generally have a minimum impact on the inflammatory response, therefore with potential for further development as antibiotic alternatives for disease control and prevention in animal agriculture. In the presentation I will highlight some of the newly identified compounds and their mechanism of action.

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From single probiotics to complex commensal microbiota: effects on immunity, enteric infections and vaccines in gnotobiotic pigs.

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The mammalian immune system has co-evolved in symbiosis with the microbiome to sense and eliminate pathogens. Residing mostly in the gut, commensal bacteria regulate immune system development, inflammation and protect against invading pathogens. Interactions between the microbiota and immune system in health and disease are very complex with the same bacterial species playing distinct and sometimes opposing roles. The knowledge of mechanisms of actions of commensal bacteria has been greatly advanced using germ-free animal models and gnotobiological techniques. Neonatal gnotobiotic (Gn) pigs resemble infants in their gut physiology, anatomy, mucosal immunity and susceptibility to human rotavirus (HRV) diarrhea. Thus, they represent a highly relevant model to elucidate how commensal/probiotic bacteria modulate HRV immunity and disease in infants.

Using the Gn pig model, we initially demonstrated that colonization with probiotics [Lactobacillus rhamnosus GG (LGG) and Bifidobacterium lactis Bb12 (Bb12)] alone, modulate neonatal immune responses to HRV, enhance HRV vaccine immunogenicity and alleviate the severity of HRV disease. Also, we showed that L. acidophilus or LGG monoassociated Gn pigs provide an excellent model to study distinct effects of individual probiotic strains at the molecular level using transcriptome profiling. We further applied the Gn pig model to compare how phylogenetically distant bacteria, LGG (gram-positive, Firmicutes) and Escherichia coli Nissle 1917, EcN, (gram-negative, Proteobacteria), individually or combinatorially modulate immune responses and HRV disease. Our results demonstrated that EcN alone protected more efficiently against HRV infection/diarrhea than LGG via potent stimulation of the innate and adaptive immune systems. Finally, we have established Gn pig models associated with complex intestinal microbiota: a defined 7-species commensal microbiota of swine origin (DMF) or a human infant fecal transplant. These models will allow for highly controlled studies of interactions among the microbiome, host immunity, diet, antibiotics, probiotics, mucosal pathogens and vaccines.

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Microbial shifts in the swine distal gut caused by the antimicrobial growth promoter tylosin

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The growth, health, and well-being of animals is now known to be intimately linked to the composition of the gut microflora (the microbiome). Antimicrobials are known to cause alterations in the composition of the microbiome and have been used extensively as growth promoters (AGPs) in animal production. The specific mechanism(s) by which AGPs act is not known but is assumed to be related to their abilities to alter the composition of the gut microbiome. The work described here was performed to document changes in the gut microbiomes of pigs receiving one AGP, tylosin, compared to untreated pigs. Out study was to determine the fecal microbiome of pigs receiving tylosin compared to untreated pigs using pyrosequencing of 16S rRNA gene libraries. The data showed microbial population shifts representing both microbial succession over time and changes in response to the use of tylosin. Quantitative analyses of sequences showed that tylosin caused microbial population shifts in both abundant and less abundant bacterial species. A total of twelve differentially abundant bacterial genera were identified. These included six abundant (>1% of the total sequences) and six less abundant genera. The six abundant genera were Prevotella, Lactobacillus, Sporacetigenium, Megasphaera, Blautia, and Sarcina, and the less abundant genera were Barnesiella, Mitsuokella, Acetanaerobacterium, Anaerosporobacter, Succinivibrio, and Eggerthella. Using heat maps to visualize the differences in the compositions of the gut microbiomes between treatment groups we found that tylosin appeared to accelerate the development of an adult gut microbiome compared to the non-treated animals. Thus, one mode of action for tylosin is to increase maturation of the gut microbiome. This data should aid in the identification of alternative strategies to improve animal health and consequently production.

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The hunt for alternatives to antibiotics in poultry: a systematic, microbial community-based approach

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The reduction of antibiotic use in animal agriculture coincides with a need for alternative approaches to enhance growth, maintain health and prevent disease. A popular tactic is to strive for this goal through the use of live microbial agents or bacterial spores. However, a systems-based approach towards understanding the mechanisms by which these agents work is lacking. Furthermore, top-down methodologies specific to production animal species are rarely considered. The purpose of this study was to undertake a comprehensive effort aimed at characterizing the succession of bacterial populations in commercial turkeys, identifying key shifts in these bacterial populations in relation to growth and development, and identifying means by which to modulate these bacterial populations without antibiotics. Characterization of several commercial and research turkey flocks revealed bacterial populations in the cecum, ileum and litter that were highly predictable and which shifted predictably during bird maturation. A key finding of this work was reproducible shifts in five bacterial species that represent microbial biomarkers of microbial succession in the ileum. The effects of several live microbial treatments were compared with antibiotic treatments on the turkey microbiome, revealing shared modulations likely relevant for their beneficial effects to birds. The relevance of sample type was also considered, demonstrating that a composite litter sample can be used in lieu of gut samples taken directly from euthanized birds. Overall, this work begins to elucidate the complexities of the turkey microbiota and ways that it can be modulated to benefit the bird and its producer.

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Commensal gut bacteria as new generation probiotics to improve gut health

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Gastrointestinal tract of humans and animals are home to trillions of microorganisms. Well established microbial community structure of these microbiota in the gut is key to growth and health. Dysbiosis of gut microbiota results in many serious enteric diseases. Clostridium difficile infection (CDI) in humans and animals is a classic example of infection caused by damage to microbiota. This talk will discuss omics based approaches that our lab is using to develop defined bacteriotherapy as a new treatment for CDI. This talk is intended to be a part of NC1202 mini symposium being organized by Dr.Gireesh Rajasekhara.

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The swine intestinal microbiota: localized responses to in-feed antibiotics

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The extensive use of antibiotics in livestock production has led to concern about the collateral effects to food safety, animal health and the host microbiota. New regulations will limit antibiotic usage and eliminate most “growth-promoting” uses of antibiotics. Our poor understanding of how antibiotics function to promote growth presents a challenge in the search for antibiotic alternatives. To improve our understanding of antibiotic effects on the commensal bacterial communities, including the proliferation of antibiotic resistance genes, we evaluated the membership and functional shifts within the microbiota of swine continually fed antimicrobials commonly used on farm. Immediately after antibiotic administration, *Escherichia coli* populations bloomed, followed by a return to basal levels two weeks later. Moreover, antibiotic-resistance genes increased in the intestinal communities after exposure to antibiotics, including genes that confer resistance to antibiotics not administered. Localized differences were detected along the intestinal tract, with *E. coli* populations increasing in the ileum and *Lachnobacterium* spp. increasing in multiple locations after antibiotics were administered. Characterizing the antibiotic effects on bacterial communities at multiple gut locations is essential to understand how the specific effects of antibiotics may impact the host. Additional research on the disturbances of beneficial bacteria is needed to mitigate the collateral impact antibiotics have on animal health and food safety.

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A highly-sensitive, field-deployable molecular assay for rapid detection of porcine epidemic diarrhea virus

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Porcine epidemic diarrhea (PED) is a highly contagious and acute enteric disease of swine characterized by diarrhea, vomiting and dehydration. The causative agent, PED virus (PEDV), is an alphacoronavirus of the Family *Coronaviridae.* In April 2013 the first case of PED was reported in the USA. Since then, this virus has killed millions of pigs in the USA and spread to neighbouring countries such as Canada, Mexico, Colombia and Peru. Many conventional and real-time RT-PCR (rRT-PCR) assays for molecular detection of PEDV have been developed. These assays require costly instrumentation and advanced technical expertise and therefore the samples need to be shipped to central laboratories for analysis. This can delay diagnosis and timely implementation of biosecurity measures needed to control virus transmission. Rapid on-site molecular diagnostic assays can overcome these impediments and facilitate early detection of PEDV. Here we report the development and evaluation of a portable, reverse transcription insulated isothermal PCR (RT-iiPCR) assay for sensitive and specific detection of PEDV. The assay detected all PEDV isolates tested, and it was as sensitive as the rRT-PCR assay currently in use at Canadian animal health laboratories. The assay did not detect transmissible gastroenteritis virus, porcine deltacoronavirus or any other viral pathogens commonly encountered in swine

operations. The reaction conditions were optimized to detect approximately 20 copies of *in vitro* transcribed PEDV RNA with a 95% probability. The assay can be performed within 1.5 hours and uses lyophilized reagents that can be shipped at ambient temperature. The instrumentation required to perform the assay is a simple, portable, relatively inexpensive, user-friendly, insulated thermal device that automatically displays the result as "+" or "-" after the end of a run without the need for user analysis.

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Bayesian estimation of sensitivity and specificity of culture- and PCR-based methods for the detection of Escherichia coli O157 in cattle feces

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Escherichia coli O157 is a foodborne pathogen that colonizes the hindgut of cattle and is shed in feces. E. coli O157 in cattle feces contaminates the hides and may be transferred onto carcasses during processing at slaughter plants. The detection of E. coli O157 in cattle feces is based on culture, immunological, and molecular methods. The objectives of this study were to generate sensitivity and specificity estimates for PCR- and culture-based methods used for the detection of E. coli O157 in cattle feces, and estimates its true prevalence using latent class Bayesian models. A total of 576 freshly voided pen floor fecal samples were collected from pens of finishing cattle in a commercial feedlot in the United States during summer 2013. Fecal samples, suspended in E. coli broth, were enriched and subjected to detection of E. coli O157 by culture (immunomagnetic separation and plating on a selective medium), conventional PCR (cPCR), and real-time quantitative PCR (qPCR). Samples were considered positive for E. coli O157 if the isolate recovered or the DNA extracted from enriched culture was positive for the rfbEO157 gene. The analyses assume conditional dependence of the PCR tests and perfect specificity for culture.

Sensitivity estimates for culture, cPCR, qPCR, and their credibility intervals (CI) were 50.4% (95% CI: 44.5-70.8%), 61.8% (95% CI: 56.1-67.8%), and 97.0% (95% CI: 94.4-98.7%), respectively. Specificity estimates were 89.7% (95% CI: 55.9-99.4%), and 54.3% (95% CI: 21.6-91.8%) for cPCR and qPCR, respectively. Conditional covariance (sensitivity & specificity) between cPCR and qPCR were 0.012 (95% CI: 0.003-0.027), and 0.028 (95% CI: -0.015 to 0.129), respectively. The true prevalence was estimated as 85.6% (95% CI: 60.5-92.5%). PCR-based tests, especially qPCR, were the most sensitive for detection of E. coli O157 in feces of naturally-shedding cattle. The qPCR was less specific compared to cPCR or culture method.

PCR-based methods offer a higher throughput and sensitivity of detection in feces but at the cost of decreased specificity. These data provide important estimates of test performance for calculating true prevalence and adjusting for test error in risk modeling.

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Optimizing & standardizing anti-STa antibody titration assay by using ovalbumin-STa fusion protein as ELISA coating antigen

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Purpose: Enterotoxigenic E. coli producing heat-labile (LT) and heat-stable (ST) toxins are the leading bacterial cause of diarrhea in humans and animals. Vaccination is considered the most practical and effective preventive approach. Current ETEC vaccine development encounters challenges including a lack of reliable assay to measure antibody responses to ST particularly STa toxin. STa-ovalbumin chemical conjugates are used as the ELISA coating antigens to titrate anti-STa antibodies. But STa-ovalbumin conjugates are very difficult to prepare.

Methods: In this study, we modified the chicken ovalbumin gene, genetically fused the STa gene to the modified ovalbumin gene for STa-ovalbumin fusion, prepared recombinant fusion protein in E. coli, and used the fusion protein as the coating antigen in ELISA to titrate STa antibodies. Different coating antigen dose (from 5 ng to 500 ng per well) were attempted in ELISAs.

Results: Results indicated that a dose of 25ng ovalbumin-STa fusion antigen coating was the optimal for anti-STa antibody titration assay.

Conclusions: These results suggested that anti-STa antibody titration assay can be standardized, leading to acceleration of ETEC vaccine development.

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A multiepitope fusion antigen of fimbrial adhesin tips of enterotoxigenic Escherichia coli (ETEC) indices broadly protective anti-adhesin antibodies

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Purpose: Enterotoxigenic Escherichia coli (ETEC) strains are the most common bacterial cause of diarrhea to young animals and children. ETEC bacteria attach to host small intestinal epithelial cells by using bacterial adhesins. It is regarded that anti-adhesin vaccines against bacterial attachment to host cells serve as the first-line of defense against ETEC diarrhea. A typical ETEC adhesin is composed of multiple copies of a major subunit and a few copies of a minor subunit tip. But it is unknown whether anti-adhesin vaccines should target at the major subunit or the minor subunit tips, and also if heterogeneous adhesin minor subunit tips can be integrated for a single antigen for developing a broadly protective anti-adhesin vaccine.

Methods: In this study, we applied the multiepitope fusion antigen (MEFA) strategy to construct a polypeptide carrying representative epitopes from adhesin tips of the 8 most important ETEC adhesins associated with diarrhea in humans, CFA/E, CFA/II (CS1, CS2, CS3), CFA/IV (CS4, CS5, CS6) and CS21, and evaluated immunogenicity of the constructed tip MEFA and potency in developing an anti-adhesin vaccine against ETEC diarrhea.

Results: Data showed that mice intraperitoneally immunized with this tip MEFA developed strong immune responses to all 8 ETEC adhesins. Moreover, induced anti-adhesin antibodies had significantly inhibited adherence of ETEC or E. coli strains expressing these 8 adhesins to Caco-2 cells.

Conclusions: These results suggested that the constructed adhesin tip MEFA can be used for developing a broadly protective anti-adhesin vaccine, and this MEFA strategy may be used in general for multivalent vaccine development.

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Adjuvanticity of double mutant heat-labile toxin (dmLT, LTR192G/L211A) of enterotoxigenic Escherichia coli (ETEC) in mouse parenteral immunizations with a toxoid fusion antigen

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Purpose: Cholera toxin (CT) of Vibrio cholera and heat-labile toxin (LT) of enterotoxigenic Escherichia coli and their derivatives have been used as adjuvants to enhance antigen-specific mucosal and systemic antibody responses. Recently, double mutant LT (dmLT, LTR192G/L211A), which possesses much reduced enterotoxicity but LT immunogenicity, is reported an effective mucosal adjuvant in oral or intranasal immunization. But adjuvant effect of dmLT in parenteral immunization has not been investigated.

Methods: In this study, we intraperitoneally (IP) immunized mice with toxoid fusion antigen 3xSTaN12S-dmLT by using dmLT or Freund's adjuvant as the adjuvant, or subcutaneously (SC) with dmLT or ISA51 as the adjuvant, and then examined adjuvanticity of dmLT in immunoregulating mouse antigen-specific immune responses.

Results: Data from this study showed that the IP immunized mice with dmLT adjuvant developed higher anti-STa and anti-LT IgG antibody responses compared to the mice immunized with the same antigen but with Freund's adjuvants. Data also revealed that the SC immunized mice using dmLT or ISA51 as the adjuvant developed similar levels of systemic anti-STa and anti-LT IgG antibody responses.

Conclusions: These results indicate dmLT is equally or more effective to immunoregulate stimulation of systemic immune responses against ETEC toxic antigens in IP and SC routes, and suggest that dmLT can be an effective adjuvant for parental vaccines against ETEC and perhaps other infectious agents.

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Effect of ascorbic acid on survival and bacterial contents in the gut contents of oreochromis niloticus

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Studies were conducted to evaluate the dietary impact of Ascorbic acid on the survival and bacterial load in the gut contents of Oreochromis niloticus. Fish stocked in the earthen ponds which were to be marketed for human consumption was taken and the Vit C as a source of ascorbic acid was added in the standard feed for evaluation. There were three tanks in replicates selected as control, treatment 1 (T1) with 3% addition and treatment 2 (T2) with 5% addition of ascorbic acid, respectively. After a ninety day trial results 100% survival was observed in all the replicates were analyzed by statistical software SAS and analysis of variance showed significant differences among the treatments. The highest microbial load was observed in control (1.12E±07 to 1.67E±06) while in the treated tanks it was observed as 1.03E±05 to 1.23E±06 in T1 and 7.10E±05 to 9.8E±05 in T2.

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Identification of potential probiotic species for growth promotion in turkey flocks.

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Light Turkey Syndrome (LTS) exclusively affects Minnesota turkey farms and is responsible for millions of dollars in losses each year. Attempts to combat LTS include probiotic use, but those used in turkey production were derived from chickens. Our objective is to identify turkey-specific probiotic bacteria capable of enhancing weight gain and feed efficiency. We sampled ceca, ilea, litter, water, and feed from two commercial flocks in Iowa and one research flock in MN. Samples were collected weekly from day-of-hatch through 12 weeks. Bacterial 16S rRNA community profiling was performed targeting the V1-3 regions and data were analyzed using mothur. Our 284 samples grouped into four community types, each with distinct species: Type I: environmental samples, *Corynebacterium*; Type II: ileal samples, *Lactobacillus*; Type III: cecal samples, *Bacteroides*; and Type IV: mixed sample types, *Faecalibacterium* and *Clostridium* XVIII. Six species within the ileal community type are identical to marker species we previously identified from MN flocks: *L. aviarius*, *L. helveticus*, *L. johnsonii*, *L. reuteri/vaginalis*, *Clostridium XI*, and Candidatus Arthromitus. *L. johnsonii* and Candidatus Arthromitus are more abundant in the brood phase of growth and the remaining marker species are more abundant in the finishing phase of growth. Combinations of select turkey-source marker species were inoculated into day-of-hatch tom poults: C1) *L. johnsonii/Clostridium XI*, C2) C1 plus *L. aviarius*, C3) Brood-phase combination, and C4) Finishing-phase combination. Bird weights and feed efficiency were monitored over 14 days and compared to a commercial probiotic. Average poult weights were consistently higher in our four treatment groups compared to the commercial probiotic group. Overall feed efficiency of the Brood Combination was comparable to the commercial probiotic and overall feed efficiency of the Finishing Combination was lower than the commercial probiotic group. We identified several turkey-source species with potential for enhanced growth promotion. When used in combination, our turkey-source marker species show increased bird weights and comparable, or better, feed efficiencies.

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ORF5 sequencing indicated PRRS strain shifting in the field

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ORF5 sequencing is a more informative method for PRRS genotyping. Kansas State Veterinary Diagnostic Laboratory (KSVDL) has been working with Abilene Animal Hospital to generate ORF5 sequencing information for PRRS management. More than 250 PRRSV strains have been sequenced at KSVDL, and phylogenetic analysis indicated that they belong to several different sub-groups with nucleic acid identity ranging from 81% to 100%. Interestingly, some strains have identity of greater than 99% to three prototypes of the four commonly used North American type PRRSV vaccine strains, although Abilene Animal Hospital has not used any of the MLVs since 1997. Phylogenetic analysis also suggests

that the genetic background of the four vaccine strains is somehow narrow, as they grouped together in a sub-cluster in one side of the un-rooted tree, and are distantly related to the majority of other field strains including contemporary strains collected in recent years. As a routine practice on an infected farm, once a predominant genotype is identified by sequencing, a "Load, Close and Expose" strategy is deployed, from which high concentration virus is collected from <10 day-old infected piglets, and sprayed onto the nose of all females. The farm is then closed down without animal trafficking. The virus is cleared in 18-26 weeks in many cases. Sequencing was also used for tracking geographic and temporal distributions of the viruses. An outbreak strain in newly purchased weaned pigs on a farm was identified to be distinct from an endemic strain of the sow farm from which the pigs were purchased. In a temporal tracking case, four PRRS strains were dominating a swine farm during 2007 and 2013, each occupies an overlapping period. Two of the four strains were more similar, with 97-99% identity to each other, and overlapped for 10 months indicating that they evolved very slowly, and no major change had occurred. Other two strains are very different from each other, with only 83-84% identity at nucleic acid level. They both are distinct from the two similar strains mentioned earlier. The ORF5 sequencing information generated at KSVDL was critically used for decision making in PRRS outbreak management at Abilene Animal Hospital.

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Detection of Actinobacillus pleuropneumoniae ApxIV toxin antibody in serum and oral fluid specimens from pigs inoculated with under experimental conditions.

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Detection of *Actinobacillus pleuropneumoniae* ApxIV toxin antibody in serum and oral fluid specimens from pigs inoculated with under experimental conditions.

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INTRODUCTION

Actinobacillus pleuropneumoniae ApxIV toxin is unique to APP and is expressed by all 15 serotypes. For this reason, the detection of anti-ApxIV serum antibodies can be used to identify APP-infected pigs. The goal of this project was to describe ApxIV antibody kinetics in serum and oral fluids specimens from animals inoculated under experimental conditions.

METHODS

Four groups of pigs (~14-weeks-old; 6 per group) were used. Animals were individually housed in order to collect individual pig oral fluids. Two weeks post-placement, animals were inoculated with APP serovars 1 (ATCC 27088), 5 (ATCC 33377), 7 (ATCC WF83) or 12 (ATCC 9799/84). Pigs were exposed intranasally (2 ml) and by direct application (3 ml) to the tonsils. Blood samples were collected weekly and oral fluids were collected daily from DPI -14 to 56. Serum samples were tested for ApxIV antibodies using a serum antibody ELISA (ApxIV Ab, IDEXX Laboratories, Inc., Westbrook, ME) and for serovar specific antibodies using LPS ELISAs (Université de Montréal). Oral fluid samples were tested using the serum ApxIV ELISA, but the protocol was optimized for the detection of ApxIV antibody in oral fluids.

RESULTS

antibodies were also positive in oral fluids.

CONLUSION

ApxIV antibody was detected in both serum and oral fluids. Antibody responses were positively associated with the strength of the clinical response. Thus, the clinical observations suggest that the appearance of ApxIV antibody is dependent either upon the severity of the infection and/or tonsil colonization.

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Genetic diversity of porcine reproductive and respiratory syndrome virus genes determined by metagenomic sequencing of clinical samples

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Porcine reproductive and respiratory syndrome virus is one of the most significant swine diseases with near worldwide distribution. In the U.S., open reading frame 5 (ORF5) is commonly sequenced to investigate viral epidemiology. To increase our understanding of PRRSV diversity and evolution, 66 genome sequences were determined directly from serum samples using viral metagenomic methodology. Phylogenetic analysis identified five, four, seven, seven and six well-supported clades for ORF2a, ORF3, ORF4, ORF5 and ORF6, respectively, which encompassed nearly all strains. This study identified more diversity in the PRRSV structural proteins than previously recognized, possibly due to direct sequencing of clinical samples as opposed to selection for growth in vitro. Since late 2013, a newly emerging PRRSV strain with ORF5 restriction fragment length polymorphism (RFLP) 174 has caused significant disease outbreaks in parts of the United States. Eleven of the 66 full genome sequences clustered with a RFLP 174 reference strain. Sequence analysis found surprisingly little genetic diversity (>95% identity) in genes encoding GP2a, GP3, GP4, GP5, M and N proteins for 11 PRRSV 174 strains, however there was considerable variability in nsp2 (74.2-100% identity) which represented two well-supported phylogenetic lineages as well as another strain with unresolved phylogeny. Three different nsp2 deletion alleles were additionally identified, two of which on are first identified here.

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Discovery and pathogenesis of porcine parainfluenza-1 in pigs in the United States

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Porcine parainfluenza virus-1 (PPIV-1) was first identified in 2013 in slaughterhouse pigs in Hong Kong, China. The pathogenesis or endemic potential of PPIV-1 has not yet been assessed nor has the virus been formally identified outside of China. Here, metagenomic sequencing was used to assemble two complete genomes of PPIV-1 from nasal swabs collected from swine in Oklahoma and Nebraska. The genomes were 91.18-96.21% identical to the previously published Chinese strains. Phylogenetic analysis of a 1720bp segment of the HN gene from 13 U.S. samples suggests moderate genetic variability between strains with nucleotide identities from 89.5-100%. Comparison of the PPIV1 U.S. and Hong Kong samples yielded an 84.8-96.4% identity. Further molecular analysis by real-time RT-PCR (qRT-PCR) identified 17 positive samples out of 279 (6.1%) lung homogenate, oral fluid, or nasal swab samples from nine states from pigs with acute respiratory disease. Eleven nursery pigs from a naturally infected herd were monitored for virus replication and pathogenesis. No clinical signs of illness were apparent however qRT-PCR detected PPIV-1 in nasal swabs from seven pigs and the lungs of one animal. In situ hybridization identified PPIV1 RNA in the nasal respiratory epithelium and trachea to a lesser extent. Serological analyses using immunoprecipitation coupled to PCR detection and ELISA demonstrated seroconversion and further analysis of 60 swine serum samples resulted in 55.0% and 63.3% seropositivity, respectively. Taken together, the results confirm the widespread presence of PPIV-1 in the United States swine herd.

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Evaluation of the use of non-pathogenic porcine circovirus type 1 as a vaccine delivery virus vector to express antgenic determinants of porcine reproductive and respiratory syndrome virus

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PCV1, first identified as a contaminant of the PK-15 cell line, is non-pathogenic and has a low prevalence in swine herds. PCV1 shares similar genomic organization with the pathogenic PCV2. Genetically modified infectious PCV1-2 can tolerate up to a 27 aa insertion in the C-terminus of the ORF2 capsid gene and generate a dual immune response against PCV2cap and the inserted epitope tag. The aim of this study was 1) to generate chimeric viruses containing neutralizing epitopes of PRRSV using the backbone of the non-pathogenic PCV1, and 2) to evaluate infectivity and immunogenicity of PCV1-PRRSVEPI chimeric viruses in vivo.

Four different B-cell linear epitopes derived from PRRSV-VR2385, including GP2 II (aa 40-51), GP3 I (aa 61-72), GP5 I (aa 35-46), and GP5 IV (aa 187-200), were cloned individually in frame into the C-terminus of the PCV1 capsid gene. In vitro infectivity and co-expression of the PCV1-capsid protein and PRRSV epitopes were evaluated by IFA. Infectivity and immunogenicity was evaluated in vivo. Serum samples were collected weekly for a period of 7 weeks. Laboratory procedures performed included qPCR for quantification of viral DNA loads, serological evaluation of IgG anti-PCV1 and anti-PRRSVEPI antibodies by IFA and ELISA respectively, and serum virus neutralization assay against PRRSV-VR2385. Four PCV1-PRRSVEPI were infectious in vitro and co-expressed PCV1cap and their respective PRRSV epitopes. Animal studies showed three PCV1-PRRSVEPI chimeric viruses produced viremia and replicated tissues. IgG anti-PCV1 antibodies were detected in serum in parental PCV1-infected pigs at 14 dpi and chimeric PCV1-PRRSVEPIGP3I, EPIGP5I and EPIGP5IV infected groups at 14, 21, and 28 dpi respectively. Anti-PRRSV-VR2385 neutralizing antibodies were detected in the PCV1-PRRSVEPIGP3I, EPIGP5I, and EPIGP5IV chimeric viruses-infected groups at 28 dpi.

Our results demonstrated that PCV1-PRRSVEPI chimeric viruses were infectious in vitro and in vivo and elicited neutralizing antibodies against PRRSV-VR2385. The results of this study provided a proof of concept for further exploring the use of the non-pathogenic PCV1 as a live virus vector for vaccine delivery.

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Agreement among sampling methods used to identify viral and bacterial pathogens in dairy calves with bovine respiratory disease (BRD)

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Bovine respiratory disease (BRD) is common in dairy calves, and antemortem sampling to identify respiratory pathogens is often undertaken. Sampling methods used include the nasal swab (NS), deep guarded nasopharyngeal swab (NPS), transtracheal wash (TTW), and bronchoalveolar lavage (BAL). However, the agreement among these tests has not been well characterized. The objective of this study was to compare the agreement of results obtained by nasal swab, NPS, or BAL with those obtained by TTW for identification of bacterial pathogens by aerobic culture, and viral pathogens by real time RT-PCR. Calves at a single facility with naturally occurring respiratory disease in the first 90 days of life were enrolled if they had a score of 5 or greater on the University of Wisconsin Calf Respiratory Scoring Chart, a rectal temperature of 103˚F or higher, and at least 2 cm of pulmonary consolidation identified by transthoracic ultrasound. Calves that had ever been treated for respiratory disease, or that had received intranasal modified live viral respiratory vaccine in the previous 30 days, were excluded. A NS, NPS, TTW, and BAL were collected sequentially from each calf using standard methods. Agreement between the TTW and the NS, NPS, or BAL was determined by calculation of the kappa statistic. Values of kappa were categorized by the following levels of agreement: less than or equal to 0.20 = poor; 0.21 - 0.40 = fair; 0.41 - 0.6 = moderate; 0.61 - 0.80 = good, and greater than or equal to 0.81 - 1.00 = very good. One hundred calves were

enrolled. Relative to the TTW, all sampling methods showed very good agreement for identification of Pasteurella multocida or Mannheimia haemolytica. In contrast, for identification of bovine respiratory syncytial virus and relative to TTW, agreement was moderate for NS, good for NPS, and very good for BAL. For identification of BCV and relative to TTW, agreement was moderate for the NS and NPS, and good for BAL. Agreement between TTW and other sampling methods differed depending on the pathogen isolated; results of BAL agreed best with the TTW for all pathogens.

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Iodine secretion in airway surface fluid following a single oral bolus of sodium iodide in calves.

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Bovine respiratory disease is a leading cause of reduced animal welfare and economic loss in beef production. Antibiotic drugs are used to counteract respiratory disease; however, antibiotic use is alarming to consumers. New methodologies for treatment and prevention of infectious diseases are being developed to reduce the use of antibiotic medications. The combination of lactoperoxidase (LPO), hydrogen peroxide (H2O2), and iodine has been shown to attenuate respiratory infections in sheep. A similar capability of the LPO/H2O2/iodine system of cattle may also exist. The objective of this study was to determine if a single oral bolus of sodium iodide would increase the iodine content of upper respiratory secretions in calves. Sixteen crossbred beef calves were divided into two equal groups by random allocation to either a treatment or control group. Blood and nasal secretions were collected immediately prior to the beginning of the study, then every 12 hours for 72 hours after the administration of a 70 mg/kg bolus of sodium iodide (treatment) or water (control). The nasal fluid of the control calves exhibited no change in the iodine content at all measured time points, Cmax = 1.41 µg/mL. The calves that received sodium iodide had a marked increase of nasal fluid iodine concentration with a peak concentration at 12 hours and Cmax = 181.40 µg/mL. A statistically significant difference ($p < 0.05$) of iodine concentration was measured at all sample collection time points except for the baseline sample. The administration of an oral bolus of sodium iodide effectively increases the iodine concentration of nasal fluid. These data indicate that iodine is likely available in concentrations suitable for incorporation into the LPO/H2O2/iodine system and that this system may be useful in treating bovine respiratory infections. Further investigation into the application and utility of increased iodine concentration of the respiratory system in cattle is needed.

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In vitro inactivation of bovine viral respiratory pathogens using an iodine-based antimicrobial system

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Bovine respiratory disease complex (BRDC) is one of the primary health concerns of cattle and the principal indication for antibiotic use in bovine medicine. Although many risk factors are involved in the pathogenesis of BRDC, infection by viral pathogens is considered to be one of the initiating steps of the disease. The combination of lactoperoxidase (LPO), hydrogen peroxide (H2O2), and iodide has demonstrated in vitro activity against human viral respiratory pathogens, but studies involving bovine viruses are lacking. Therefore, the purpose of this study was to determine the in vitro susceptibility of parainfluenza 3 virus (PI3), bovine herpesvirus-1 (BoHV1), and bovine viral diarrhea virus (BVDV) to the LPO/H2O2/iodine system. Stock cultures of each virus were incubated with LPO, H2O2, and sodium iodide at a concentration of 0, 10, 100, or 250 µM for 5, 15 or 60 minutes. Each assay was repeated in triplicate. Virus titration was performed by direct visualization of cytopathic effect in cultured cells. A dose-dependent decrease in viral concentration was observed for PI3, with decreased concentrations seen in all samples at even the lowest concentration of iodine included. For BoHV1, significant reduction in viral titer was seen at 100 µM iodine concentration. Of the 3 viruses, BVDV showed the least susceptibility to the system with no decrease in titer observed at even the highest concentrations of iodine. Titers were not affected by incubation time. This study indicates the LPO/H2O2/iodine system may hold value in the prevention of respiratory viral infection and the subsequent risk of BRDC.

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Sodium iodide inactivates Rhodococcus Equi in vitro

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Sodium iodide inactivates *Rhodococcus Equi in vitro*

Rhodoccocus equi causes a granulomatous pneumonia in foals which is a major cause of morbidity and mortality and costs the equine industry millions of dollars for treatment. Sodium Iodide has been used to treat granulomatous infections in humans and veterinary species for over a hundred years, however its mechanism of action is still not understood. Iodide also plays a role in preventing infections on epithelial surfaces. In humans, oral supplementation with iodine increases the levels of hypoiodous acid on respiratory epithelial surfaces which has potent anti-microbial and anti-viral effects. No studies have been done to look at the susceptibility of common equine pathogens to this enzyme system. There are two potential roles for NaI in the augmentation of the innate immune response; one is as a treatment for granulomatous infections and the other is to prevent infection via epitheial surfaces. *R. equi* infections fulfill both of these circumstances. The susceptibility of *R. equi* to inactivation by hypoiodous acid (HOI), was determined by generating HOI in vitro using LPO , sodium iodide and H2O2. Varying concentrations of NaI were used, and controls were run in parallel. Subsamples were removed at various time points to determine bacterial load via standard plate counts. At all concentrations of NaI tested (25uM-250uM), R. equi was killed by the complete reaction products. Surprisingly, NaI alone at higher concentrations (above 50uM) inactivated *R. equi* in vitro. NaI shows exciting promise as a preventative for respiratory *R.equi* infections.

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Sodium iodide inactivates Manheimia hemolytica and Bibersteinia trehalosi in vitro.

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Sodium iodide inactivates *Manheimia hemolytica* and *Bibersteinia trehalosi in vitro*.

Bovine respiratory disease is an important cause of economic loss in the beef industry accounting for 31% of mortality in calves three weeks of age or older. The airway epithelium has several innate defense mechanisms to protect the health of bovine lungs; secreted antimicrobial peptides neutralize pathogens, mucus and ciliated epithelium trap and remove pathogens from the respiratory tract, and lactoperoxidases (LPO) and hydrogen peroxide (H2O2) excreted by the epithelium react with halide ions to form hypohalous acids. It was recently shown that when iodide is utilized as the halide ion the resulting hypoiodous acid has potent antiviral activity against human respiratory syncytial virus and adenovirus *in-vitro*. The objective of this study was to determine if two important bovine bacterial respiratory pathogens, *Manheimia hemolytica* and *Bibersteinia trehalosi (*formerly *M. hemolytica* biotype T*),* are susceptible to inactivation by the LPO/H2O2/halide system that is present in bovine airways. The susceptibility of these pathogens to inactivation by hypoiodous acid was determined by generating HOI in vitro using LPO, sodium iodide (NaI) and H2O2. Concentrations of NaI from 25 µM to 250 µM were tested, and controls were run in parallel. Subsamples were removed prior to addition of H2O2 (catalyst) and at 5 and 30 minutes. Bacterial killing was assessed via standard plate counts. At all concentrations of NaI, both *M. hemolytica* and *B. trehalosi* were killed immediately by the complete reaction. Surprisingly, NaI alone at higher concentrations (above 50uM) inactivated both pathogens *in vitro*. NaI shows exciting promise as a preventative strategy for bovine respiratory disease which may also reduce antimicrobial use in food producing animals.

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Zelnate™: a novel approach to BRD management in cattle

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Bovine Respiratory Disease (BRD) inflicts a major economic impact on U.S. beef and dairy industries. The objective of these studies was to evaluate the efficacy of a novel non-antimicrobial, non-coded DNA immunostimulant formulated in a liposome carrier (Zelnate™; ZEL) in the management of BRD. Development of ZEL consisted of a *Mannheimia haemolytica* (Mh) disease challenge model where lung lesions or mortality were the primary outcome. On Day 0, 3-4 month old Holstein steer calves were intratracheally challenged with Mh inoculum. On Day 5 (or at the time of premature death) lung lesions were estimated by study personnel blinded to treatment. In two separate studies, ZEL was administered on either Day 0 (Study 1) or Day 1 ([24 hours after Mh challenge] Study 2). In Study 1, a significant reduction (p=0.0273) in lung lesions was observed among calves administered ZEL compared to the negative control. In Study 2, a significant reduction (p=0.0394) in mortality was observed among cattle receiving ZEL relative to the negative control within the Mh disease model. The studies indicated that ZEL significantly reduced lung lesions (Study 1) and mortality (Study 2) in comparison to the negative control. These data confirm that Zelnate™ is a non-antibiotic option to aid in the management of BRD.

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Evaluation of response to vaccination on the feedlot performance of weaned calves.

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The bovine respiratory disease complex (BRDC) is one of the most costly diseases for beef production in the U.S. Vaccination protocols to prevent BRDC are commonly used in beef herds. Unfortunately, some vaccines upon administration may negatively impact feed intakes, and feeding behavior. Previously vaccinated beef steers (356.21±35.85kg, n=76) were blocked by weight and randomly assigned to one of four treatments(n=19 per treatment), treatment one was a 2 ml sterile saline injected subcutaneously in the left side of the neck, treatment two was a MLV vaccine (IBR, PI3, BRSV, BVDV type 1 and 2) in combination with a Mannheimia haemolytica toxoid, treatment three was an intranasal MLV vaccine (IBR, PI3, BRSV) along with a MLV vaccine (type 1 and 2 BVDV) in combination with a Mannheimia haemolytica toxoid, and treatment four was a MLV vaccine (IBR, PI3, BRSV, BVDV type 1 and 2) in combination with a Mannheimia haemolytica toxoid plus an intransal MLV vaccine (IBR, PI3, BRSV). The objective was to determine the effects of vaccination protocols on the acute phase inflammatory response, adaptive antibody response, feeding performance and feeding behavior of weaned calves. All treatments were administered as per label. Individual feed intake and feeding behavior was monitored using the Insentec roughage intake control system (Insentec, B. V., Marknesse, the Netherlands). Calves were vaccinated on day 0 of the trial, and weights and blood samples were collected on d 0, 1, 3, 6 and 28 of the trial. Haptoglobin, an acute phase protein, as well as antibody titers for bovine respiratory syncytial virus (BRSV) and infectious bovine rhinotracheitis (IBR), were used as a proxy to measure vaccine response. All vaccines initiated an inflammatory response ($P < 0.001$). Treatments two and four induced an increase in serum antibodies by d 28. Feed intake and behavior were unaffected by the use of vaccines. ADG tended to be higher in treatment two ($P = 0.06$). In well managed, properly immunized herds, vaccination against BRD pathogens can stimulate antibody production without negative effects of the acute phase inflammatory response on feed intake, feeding behavior and performance.

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Evaluation of on-arrival vaccination and deworming on stocker cattle health and growth performance

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The effect of vaccinating and deworming stocker cattle at arrival is poorly documented and potentially detrimental. Our objective was to evaluate the effect of on-arrival vaccination and deworming on bovine respiratory disease (BRD) incidence, mortality, and growth of stocker calves. Calves (n=80) received from an order-buyer were stratified by d-3 weight and fecal egg count into 20 pens of 4 animals each. Pens were randomly assigned to treatments in a 2x2 factorial design to test vaccination at arrival (d0 modified-live BRD and clostridial vaccine or not) and deworming (d0 oral fenbendazole and levamisole or not). Body weight and blood was collected days 0, 14, 28, 42, 56, 70 and 85. Fecal egg counts were measured days -3, 28, 56, and 85. Clinical signs of BRD (depression, anorexia, rapid respiratory rate, cough, nasal discharge, and rectal temperature of ≥104F) were monitored daily. Treatment effects on BRD incidence, mortality, and growth were tested using Poisson, logistic, or linear regression, respectively ($\alpha \leq 0.05$). BRD incidence was greater for calves with d0 vaccination (RR=3.2), high (≥104F) fever at day 0 (RR=6) and higher d-3 FEC (RR=1.2 per 100 epg). Mortality was greater for d0 vaccination (OR=8.3) and high fever (OR=41.6). Growth was lower for d0 vaccination (-10.3 lbs), moderate (103-103.9F) and high fever (-24.1 lbs and -16 lbs, respectively), and number of times treated for BRD (-17.5 lbs/treatment). Deworming at arrival was not found to be significantly associated with BRD morbidity, mortality, or weight gain. Health and growth performance of stocker calves may be adversely affected by vaccination at arrival, as well as degree of parasitism and fever at arrival.

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Comparison of the immune response to subcutaneous or intranasal modified-live virus booster vaccination in young beef calves that were primed with intranasal vaccine

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Vaccination is utilized in an attempt to prevent bovine respiratory disease in cattle; however, maternally derived antibodies have been shown to interfere with the immune response to vaccination in young calves. The objective was to compare the serum neutralizing antibody titers (SNA) to BHV1 and BRSV, and nasal mucosal BHV1-specific IgA (BHV1-IgA) following intranasal (IN) or subcutaneous (SC) booster vaccination 60 days after IN priming of young calves using a modified-live virus (MLV) vaccine. Twenty-four beef calves (3-25 days old) were prime vaccinated against BHV1, BRSV and PI3V IN (Inforce-3®, Zoetis Animal Health) and 60 days later were randomly assigned to receive a booster of the same vaccine either IN (n= 12) or SC (n= 12). Blood and nasal secretion samples were collected on days -60 (priming vaccine day), -14, 0 (booster vaccine day), 14, 21, 28, and 60 for determination of total serum IgG (only at d-60), SNA to BHV1 and BRSV, total mucosal IgA, and mucosal BHV1-IgA concentrations. On d-60, total serum IgG concentration was similar between groups. Calves had high SNA to BRSV and BHV1 at d-60, which decreased on d-14 and d0. Intranasal, but not SC, booster vaccination significantly increased the SNA to BSRV on d14, 21 and 28 compared to d0 ($P < 0.05$). Both groups had a sustained increase in nasal BHV1-IgA concentration after priming and booster vaccinations. By comparing the fold change of nasal BHV1-IgA on d21, two patterns of response were observed in IN group; calves with high concentrations of nasal BHV1-IgA at the time of booster did not show clear recall response to IN booster vaccination, but those with lower concentrations of nasal BHV1-IgA at the time of booster vaccination showed a strong (≥ 8 fold increase) recall titer at d21 and d28. In contrast, a significant nasal BHV1-IgA response was observed in the SC booster group regardless the concentration of BHV1-IgA in nasal secretions at d0. In conclusion, IN booster significantly induced SNA to BSRV in young calves IN primed with MLV vaccine; and both, IN and SC, booster increased the BHV1-IgA titers in nasal secretions. The amount of IgA present at the time of the IN booster vaccination affected the BHV1-specific nasal antibody recall response.

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Immune responses, clinical and pathological outcomes in challenged calves immunized with a subunit vaccine for BRSV and H.somni

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Dual infection with BRSV and H. somni causes more severe respiratory disease than single infection with either pathogen. Vaccination with the whole virus or whole bacteria has been associated with enhanced disease. Our hypothesis was that immunization with a single immunogenic protein from each pathogen would create a protective immune response superior to that of killed whole pathogen vaccines.Three groups of Holstein dairy calves were immunized twice with recombinant BRSV nucleoprotein (NP) and H. somni IbpADR2 in Quil A, formalin inactivated BRSV in alum plus a commercial H. somni bacterin (Somnivac), or adjuvant control. All animals were challenged with BRSV (day 0) and with H. somni (day 6). Clinical signs were scored daily and BRSV shedding was monitored by qRT-PCR. Protection was determined by clinical signs, IgG1, IgG2, IgA and IgE pathogen specific antibody responses, lymphocyte expression of CD8, CD4+CD25+FoxP3+(Treg),intracellular IFNγ,and IL-4, and pathology. Clinical disease was significantly more severe in calves immunized with FI BRSV/Somnivac than those immunized with the subunits or controls. Quantitative evaluation of lung lesions in six compartments showed significantly less pathology for NP/IbpADR2 vaccinated calves compared with adjuvant controls in bronchioles, alveoli and septae, with almost significant differences in pleural pathology. Serum BRSV and H.somni specific IgG1, IgG2, and IgE values were highest in FI BRSV/Somnivac calves. However, serum IgG1 and IgG2 responses to IbpADR2 and and IgG1 responses to BRSV NP were highest in calves vaccinated with the subunits, implying that these specific responses were related to protection. IgE responses to BRSV and H. somni were highest in the FI BRSV/Somnivac group suggesting that

this may have been at least partially responsible for the increased clinical signs in this group. Both vaccinated groups showed significant increases in CD4+ lymphocytes with intracellular interferon γ. Clinical, pathological, and immunological data indicate that a subunit vaccine consisting of BRSV NP and H.somni IbpADR2 protein provides protection from virulent challenge without disease enhancement.

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Histophilus somni increases expression of antiviral proteins in bovine respiratory epithelial cells.

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We previously showed that bovine respiratory syncytial virus (BRSV) followed by *Histophilus somni* causes more severe bovine respiratory disease and a more permeable alveolar barrier in vitro than either agent alone. Microarray analysis revealed that treatment of bovine alveolar type 2 (BAT2) epithelial cells with BRSV followed by *H. somni* concentrated culture supernatant (CCS) stimulated greater up-regulation of cytokines, chemokines and matrix metalloproteinases than treatment with either agent alone, consistent with viral bacterial synergy. However, treatment of these cells with *H. somni* CCS alone stimulated increased expression of four antiviral protein genes as compared with BRSV infection or dual treatment. This suggested that inhibition of viral infection, rather than synergy, may occur if the bacterial infection occurred before the viral infection. Viperin (or radical S-adenosyl methionine domain containing 2 - RSAD2) and ISG15 (IFN-stimulated gene 15) were most up-regulated. CCS dose and time course for up-regulation of viperin protein levels were determined in treated bovine turbinate (BT) upper respiratory cells and BAT2 lower respiratory cells by Western blotting. Treatment of BAT2 cells with *H. somni* culture supernatant before BRSV infection dramatically reduced viral replication as determined by qRT PCR, supporting the hypothesis that the bacterial infection may inhibit viral infection. Studies of the role of the two known H. somni cytotoxins showed that viperin protein expression was induced by endotoxin (lipooligosaccharide) but not by IbpA, which mediates alveolar permeability and H. somni invasion. We then showed that asymptomatic carrier strain 129Pt, which does not produce IbpA, attached to BT cells and induced a strong viperin response in vitro. Thus colonization of the bovine upper respiratory tract with an asymptomatic carrier strain lacking virulence may decrease viral infection and the subsequent bacterial respiratory infection in vivo.

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Inhibition of *Pasteurella multocida* biofilm formation by capsular polysaccharide, and interaction with *Histophilus somni* in a polymicrobial biofilm

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Histophilus somni and *Pasteurella multocida* both contribute to bovine respiratory disease. Following experimental challenge of calves with *H. somni*, and during natural infection, *P. multocida* is often isolated with *H. somni*. *H. somni* also forms a prominent biofilm during infection of the bovine cardio-respiratory system. Therefore, we sought to examine biofilm formation by *P. multocida* and the interrelationship between *H. somni* and *P. multocida* in a polymicrobial biofilm. Biofilm formation, determined by crystal violet staining and confocal scanning laser microscopy, by *P. multocida* was found to be inversely correlated with capsule formation. However, biofilm formation was significantly increased after 5 days serial passage of the parent or by mutants lacking capsule, and several genes involved in carbohydrate regulation and modification were upregulated, as determined by reverse transcriptase PCR. The P. multocida biofilm consisted of both protein and glucan. *P. multocida* did not auto-agglutinate, but when grown with *H. somni*, which auto-agglutinates well, *P. multocida* auto-agglutination increased. Both good biofilm-forming and poor biofilm-forming strains of *P. multocida* grew within the *H. somni* biofilm. The addition of *P. multocida* to the *H. somni* biofilm did not significantly increase the biofilm matrix, indicating an opportunistic relationship. Two to 3 days after the addition of *P. multocida* to the *H. somni* biofilm, little or no *H. somni* could be recovered from the biofilm, but *P. multocida* persisted. However, *H. somni* was not inhibited by *P. multocida* when they were grown together on blood agar. While carbohydrate content increased over time in the *H. somni* biofilm, carbohydrate content peaked after 2 days incubation of the *P. multocida* and polymicrobial biofilms, and then greatly diminished. Following *H. somni* respiratory challenge of 6 calves raised in isolation from birth, *P. multocida* was isolated with *H. somni* from 5 of them. Distribution of each species in lung tissue is being examined by fluorescence in situ hybridization. We conclude that encapsulated *P. multocida* may co-inhabit and exploit the *H. somni* biofilm to persist in the bovine lower respiratory tract.

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Genetic engineering of cattle that produce leukocytes resistant to Mannheimia haemolytica leukotoxin

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Mannheimia haemolytica is an important pathogen of bovine respiratory disease complex (BRDC). The leukotoxin (Lkt) produced by M. haemolytica binds to the intact signal peptide of CD18 expressed on bovine leukocytes, and lyses them. Previously, we have shown that the signal peptide of ruminant CD18 is not cleaved due to the presence of 'cleavage-inhibiting' glutamine (Q), instead of 'cleavage-conducive' glycine (G), at amino acid position 5 upstream of the cleavage site. Site-directed mutagenesis of Q to G results in cleavage of the signal peptide and abrogation of cytolysis of transfectants expressing bovine CD18 with the Q(-5)G mutation. We hypothesized that genetically engineered cattle that produce leukocytes expressing CD18 containing the Q(-5)G mutation will be resistant to Lkt, and hence less susceptible to BRDC. As the first step towards developing calves resistant to Lkt, fibroblast cell lines were developed from a 45 day-old fetus. Fibroblasts were co-transfected with CD18 oligonucleotide (120bp) carrying the Q(-5)G mutation and mRNA of the zinc finger nuclease (ZFN) custom-designed to bind and induce double-strand break of the DNA in the vicinity of the amino acid to be mutated. Cells were cloned by limiting dilution and

genomic DNA was extracted from single clones and tested for the presence of the Q(-5)G mutation by 'junction PCR'. Somatic cell nuclear transfer (SCNT) was performed with one of the selected clones with bi-allelic modification of CD18. Embryos resulting from SCNT were transferred to surrogate cows. A 125-day-old fetus was removed by Cesarean section. Flow cytometric analysis revealed the absence of signal peptide on CD18 expressed on the leukocytes of the genetically-modified fetus. More importantly, the leukocytes were resistant to Lkt-induced cytotoxicity. Sequencing analysis of CD18 confirmed the presence of the codon encoding G (GGA) instead of the codon encoding Q (CAG) in genetically-modified CD18. Surrogate cows pregnant with the genetically-modified calves are expected to deliver the calves early next year. This technology should provide a means of developing cattle resistant to M. haemolytica Lkt, and hence less susceptible to BRDC.

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Mannheimia haemolytica leukotoxin is cytotoxic even in the absence of acylation.

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Mannheimia haemolytica causes pneumonia in domestic and wild ruminants. An exotoxin produced by this bacterium is cytotoxic to all subsets of ruminant leukocytes and hence known as leukotoxin (Lkt). Based on the fact that Lkt-deletion mutants cause reduced mortality and milder lung lesions, Lkt is accepted as the most important virulence factor of this bacterium. Lkt belongs to the repeats-in-toxin (RTX) family of toxins produced by a group of Gram-negative bacteria. Previous studies have reported that RTX toxins require a fatty acid acylation step in order to become cytotoxic. In M. haemolytica Lkt, this acylation step is mediated by a transacylase protein (LktC) encoded within the Lkt operon. An LktC mutant that we (Highlander) developed previously was only partially attenuated in its virulence for cattle. Therefore the objective of this study was to elucidate the role of LktC-mediated acylation in Lkt-induced cytotoxicity. We performed this study in bighorn sheep (Ovis canadensis) (BHS), since they are highly susceptible to M. haemolytica infection. The LktC mutant caused fatal pneumonia in 40% of inoculated BHS. Lkt from the mutant was cytotoxic to BHS PMNs in an in vitro cytotoxicity assay. Flow cytometric analysis of mutant Lkt-treated PMNs revealed the induction of necrosis. Scanning electron microscopic analysis showed the presence of pores and blebs on mutant-Lkt-treated PMNs. Mass spectrometric analysis confirmed that the mutant secreted an unacylated Lkt. Taken together, these results suggest that acylation is not necessary for the cytotoxic activity of M. haemolytica Lkt but it enhances the potency of the toxin. Therefore, the use of LktC mutants as vaccine candidates warrants further studies.

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Development of a nucleotide polymorphism-based typing method for *Mannheimia haemolytica* and identification of a subtype that associates with bovine respiratory disease

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Bovine respiratory disease complex (BRDC) is a serious health and economic problem that costs the United States cattle industry over a billion dollars annually. *Mannheimia haemolytica* is a major bacterial component of BRDC. *M. haemolytica* is normally found in the upper respiratory tract of cattle, however, when the animals are stressed, it can invade the lower respiratory tract and cause severe fibrinonecrotic pneumonia and death. *M. haemolytica* have been "typed" by multiple methods including capsular serotyping and pulsed-field gel electrophoresis (PFGE). Both of these methods have been used to show that *M. haemolytica* are diverse and that not all members of the species associate with bovine respiratory disease. However, neither capsular serotyping nor PFGE effectively portray the genetic relatedness of *M. haemolytica* strains. Accordingly, the goals of this work were to 1) develop a nucleotide polymorphism-based typing method for *M. haemolytica* that identifies genetic subtypes by their evolutionary relatedness, 2) test *M. haemolytica* subtypes for an association with bovine respiratory disease, and 3) identify a minimal set of nucleotide polymorphisms that efficiently detects or "tags" the genetic subtypes that can be freely used without restriction. Accordingly, the genomes of 1,134 *M. haemolytica* isolates that collectively represent the upper and lower respiratory tracts of cattle either clinically-ill with respiratory disease or asymptomatic, 35 U.S. states, 5 Canadian Provinces, and the years 2002-2011, and 2013 were sequenced to a level of 10X per isolate genome. Over 16,000 nucleotide polymorphisms were identified across conserved regions of the genome and used for phylogenetic analyses and subtype identification. Two major clades were identified, of which one was found to associate with bovine respiratory disease. Less than 300 nucleotides effectively tag all the genetic variation identified in the study. This new typing system can be used to identify and track *M. haemolytica* genetic subtypes, including those that associate with bovine respiratory disease.

Vector-Borne and Parasitic Diseases

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Vaccination with an attenuated mutant of Ehrlichia chaffeensis induces pathogen-specific CD4 T cell immunity and protection from wildtype challenge in a canine host

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Ehrlichia chaffeensis is an obligate intracellular gram-negative bacteria and the causative agent of human monocytic ehrlichiosis (HME). E. chaffeensis has recently emerged as a frequent cause of severe and fatal tick-borne infection in people in North America. The reservoir host for E. chaffeensis is the white-tailed deer, while humans, dogs, coyotes and goats are regarded as common incidental hosts. Currently there is no vaccine available for use against E. chaffeensis, and treatment options are limited to a single class of antibiotics. We have recently established a protocol for transposon mutagenesis in Ehrlichia species; and have developed a stable mutant of E. chaffeensis in Ech_0660 gene whose growth is attenuated in vivo in the vertebrate host. We show here that vaccination with Ech_0660 protects dogs from secondary, needle-inoculated or tick-transmitted challenge with wildtype E. chaffeensis, suggesting that Ech_0660 mutant serves as an ideal candidate for vaccine development. The immune responses that correlate with control and clearance of E. chaffeensis, and protection from a secondary challenge, are poorly defined in the physiologic host. We demonstrate that dogs vaccinated with Ech_0660 mutant and challenged with wildtype E. chaffeensis mount a

pathogen-specific antibody response and a robust CD4 T cell response; with significant production of the Th1 cytokine, IFNγ, and the Th17 cytokine, IL-17. In our study, dogs infected with E. chaffeensis, with or without Ech_0660 mutant vaccination, did not mount a significant CD8 T cell response. Together, our results suggest that the attenuated mutant Ech_0660 represents a promising vaccine candidate for use against E. chaffeensis infection in humans and animals; and that pathogen-specific antibody and CD4 T cell responses are likely critical for protection from E. chaffeensis infection in the incidental host.

Vector-Borne and Parasitic Diseases

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Development and validation of real-time PCR assay for canine Lyme disease

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Borrelia burgdorferi is the causative agent of Lyme disease, which is a zoonotic multisystem disease affecting people and many vertebrates including dogs. The pathogen is a Gram negative spirochete transmitted by Ixodes species, including I. scapularis and I. pacificus ticks. Clinical signs in animals include fever, arthritis and less commonly glomerulonephritis, uveitis, myocarditis and neurologic signs. A real-time PCR assay has been developed targeting the conserved flagellin gene sequences of B. burgdorferi. In this assay, the target gene segment of the organism is amplified by PCR and identified by real-time TaqMan probe-based method. The assay was evaluated for its use in detecting B. burgdorferi infections in canine blood samples. A recombinant plasmid containing the target gene segment was prepared and serial dilutions of the plasmid were used for defining the analytical sensitivity of the assay; an equivalent to one copy plasmid was detected. DNA samples from other tick borne pathogens of dogs were also assessed to define the specificity and the assay showed no amplification with genomic DNAs of Ehrlichia chaffeensis, E. canis, Anaplasma phagocytophilum, A. platys, and Rickettsia rickettsii. This assay has been validated on plasma samples collected from 24 dogs testing positive for Lyme disease by commercial Borrelia antibody detection kits. Four out of the 24 seropositive samples tested positive with the real-time PCR assay. These samples were further confirmed by nested PCR and sequencing. In addition, 37 seronegative samples also tested negative with this real-time assay. Even though B. burgdorferi is rarely found in circulation of infected dogs, the current assay could detect the presence of the pathogen in 16% of seropositive samples. The assay could be adapted for other clinical specimens like synovial fluid, cerebrospinal fluid and skin biopsies. Together, the data demonstrate that the real-time PCR assay can be useful for diagnosing B. burgdorferi infections in dogs.

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Comparison of an alternative diagnostic sampling technique for Tritrichomonas foetus in cattle

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Bovine trichomoniasis is emerging as a major concern in the beef industry. Recent advancements in PCR diagnostics have increased the ability to detect the disease in asymptomatic bulls. However, the greatest limitation is proper collection of an adequate sample. Furthermore, the low repeatability of most sample collection techniques can cause confusion and misdiagnosis. The aim of the study was to validate a sample collection technique that increased sensitivity and was easier and safer to collect than preputial scraping. Eighty commercial bulls of unknown infection status, were sampled for detection of Tritrichomonas foetus using two different collection methods: 1) traditional preputial scraping with a dry insemination pipette (TPS) and 2) penile preputial swabbing (PPS). TPS samples were taken by vigorously scraping preputial/penile mucosa using a rigid insemination pipette while applying negative pressure. PPS samples were obtained by briskly swabbing the penile and preputial mucosa with gauze sponge during full extension of the penis. All samples were processed using InPouch™ TF media and submitted under similar conditions for PCR testing at ISU Veterinary Diagnostic Laboratory. Positive PCR results were observed in 28/80 (35%) bulls using TPS technique, however 31/80 (39%) were positive using PPS technique. Sensitivity was determined with web based application utilizing R software. The Newton-Raphson algorithm predicted the sensitivity of the TPS method was 0.897 (CI 0.637-0.978) and the sensitivity of the PPS was 0.962 (CI 0.774-0.995). This data indicates that the PPS technique is a reliable alternative to the TPS method.

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Development of a recombinant subunit vaccine for Rift Valley fever

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Rift Valley fever (RVF) is a zoonotic disease that causes severe epizootic disease in ruminant livestock, characterized by mass abortion and high mortality rates in younger animals. The lack of a fully licensed vaccine in the US has spurred increased demand for the development of an efficacious, safe and DIVA (differentiating infected from vaccinated animals) compatible vaccine. Consequently, the present study was performed in two phases to develop a glycoprotein-based subunit vaccine for RVF: (i) experimental inoculation of sheep as a susceptible host species to develop a challenge model for RVF, and (ii) evaluation of the efficacy of an experimental recombinant subunit vaccine candidate. In the animal inoculation study, a group of sheep was inoculated subcutaneously with 1 x 10^6 PFU of either SA01-1322 (SA01) or Kenya-128b-15 (Ken06) strains of RVF virus (RVFV). All infected sheep manifested early-onset viremia accompanied by a transient increase in temperatures. The sheep seroconverted and developed time-dependent increases in virus neutralizing antibody titers. The Ken06 strain manifested higher virulence compared to SA01 by inducing more severe liver damage demonstrated by higher liver enzyme activity. Liver lesions were positive by immunohistochemistry for RVFV antigen. Full-genome sequence analysis revealed significant amino acid variations between the two virus strains, which potentially could account for the observed phenotypic differences (virulence). In a subsequent vaccine efficacy study, a group of

sheep was vaccinated subcutaneously with RVFV glycoprotein-based subunit vaccine candidate and subjected to virulent challenge with 1 x 10^6 PFU of the Ken06 strain. The experimental vaccine candidate elicited high virus neutralizing antibody titers and conferred complete protection in all sheep, as evidenced by prevention of viremia, fever and by histopathological examination. The vaccine candidate is DIVA-compatible and represents a promising platform for the prevention and control of RVFV infections in susceptible hosts.

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Fish mucus; a physical barrier to pathogens

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Abstract

Fish mucus provides primary immunity against the pathogen invasion. It reduces pathogen access due to the downward movement along lateral sides of the fish and its various sprawling edges and sides. Studies were aimed to investigate the probable role of mucus in reception and/or repulsion of *Lernaea* the most common parasite in Indian and Chinese major carps and a potential threat to sustainable fish production. Grass carp (*Ctenopharyngodon idellus*), silver carp (*Hypophthalmichthys moiltrix*), rohu (*Labeo rohita*), mrigal (*Cirrhinus mrigala*), thaila (*Catla catla)* and common carp (*Cyprinus carpio*) averaging 830 ± 316 gm each was collected from grow out ponds provided with all the requirements for normal fish growth. Collected fish samples were bathed in potassium permanganate (8.0ppm) for collections of contamination free mucus. Total protein contents were determined by Bradford Micro Assay technique using Bovine Serum Albumin (BSA) as standard. Size categorization of the proteins was achieved by Polyacrylamide Gel Electrophoresis (SDS-PAGE) and Lectin Activity was measured by determining hemagglutination (HA) titer. Alkaline phosphatase activity was determined by Alkaline Phosphatase Test. Protein concentrations were the highest in *Ctenopharyngodon idella* (3.29 ± 0.13 mg/ml) and *Catla catla* (3.02 ± 0.57 mg/ml) while it was the lowest in *Cyprinus carpio* (1.80 ± 0.09). Protein concentrations in *Cirrhinus mrigala* and *Labeo rohita* fell in between these two extremes. Considering protein profiles mucus samples from *Catla catla* contained the highest molecular weight proteins (100 kDa) while *Cyprinus carpio* has one unique protein band of 14.13 kDa not present in any other species in current setup. Lectin activity was the highest in *Ctenopharyngodon idellus* (10^9) indicative of low resistance while it was the lowest (2^1) in *Hypophthalmichthys molitrix* which was completely parasite free. Alkaline phosphatase level was the highest in *Catla catla.it* reveals that protein concentrations, lectin activity, protein profiles and level of alkaline phosphatase are good indicators of resistance capability of fish.

Key words: carps; Lernaea; Lectin activity; Alkaline phosphatase

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Development of a generic Ehrlichia FRET-qPCR and investigation of ehrlichioses in domestic ruminants on five Caribbean islands

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A generic *Ehrlichia* FRET-qPCR was designed to detect the five major *Ehrlichia* species in a single FRET-qPCR reaction. Using reference organisms, our generic *Ehrlichia* FRET-qPCR reliably detected the five most generally recognized *Ehrlichia* spp., namely *E. ruminantium*, *E. canis*, *E. chaffeensis*, *E. muris* and *E. ewingii*. It also detected other *Ehrlichia* described in domestic ruminants, mainly *E. ovina*, the Panola Mountain *Ehrlichia*, and *Ehrlichia* sp. BOV2010. Melting point analysis revealed 4 distinct groups of *Ehrlichia*. When the FRET-qPCR was used on domestic ruminants from five Caribbean islands, we found 12% (134/1,101) positive: cattle (76/385; 20%), sheep (45/340; 13.2%) and goats (4%; 13/376). Melting point analysis and sequencing of the generic *Ehrlichia* FRET-qPCR products and those of nested PCRs for the citrate synthase gene (*gltA*) of *Ehrlichia* spp. showed the *Ehrlichia* we detected were *E. canis* or very closely related organisms.

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Molecular approaches in understanding *Ehrlichia* pathogenesis, host-pathogen interactions and in developing vaccines

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Ehrlichiosis in people and dogs are caused by three tick-borne rickettsials of the genus *Ehrlichia; E. chaffeensis, E. ewingii* and *E. canis*. *Amblyomma americanum* is the transmitting tick vector for *E. chaffeensis* and *E. ewingii*, while primarily *Rhipicephalus sanguineus* ticks transmit *E. canis*. Canine and human ehrlichiosis is frequently documented from the US and many parts of the world. As *Ehrlichia* species primarily infect phagocytes (monocytes/macrophages and granulocytes), they can suppress host defenses and significantly impair the health of animals and people. The infections also persist in infected hosts recovered from a clinical disease for several years; subclinical infections can increase the susceptibility to secondary infections and can also cause reversion of a clinical disease. Further, the persistently infected vertebrates, such as dogs, serve as reservoirs of infection for transmission to healthy dogs and humans. Very little is known about how the rickettsials are able to overcome host clearance and persist in both vertebrate and tick hosts. Our research over the past two decades has focused on assessing the pathogen molecular structure, host responses, and the impact of vector and host cell environments in causing the diseases and persistent infections by *Ehrlichia* species. Our research is mostly carried out using *E. chaffeensis* as the model system and by conducting research using *in vitro* and *in vivo* systems involving animal and tick infections. Our recent research in understanding how the pathogens regulate their gene expression in support of adaptation to duel host environments will be discussed. Further, our recent progress in mutagenesis studies in support of understanding disease pathogenesis and vaccine development and their implications in controlling various tick-borne diseases in dogs, humans and other vertebrates will be discussed.

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Sequence determinants spanning -35 motif and AT-rich spacer sequences impacting *ehrlichia chaffeensis* sigma 70-dependent promoter activity of two differentially expressed p28 outer membrane protein genes

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Ehrlichia chaffeensis is an obligate intracellular tick-borne pathogen that replicates in both tick and vertebrate hosts. The ability of such pathogens to regulate gene expression in tick and vertebrate hosts is currently poorly understood. In prokaryotes, transcriptional specificity of promoters is controlled by sigma factors. E. chaffeensis genome contains genes for only two sigma factors; sigma 32 (σ^{32}) and sigma70 (σ^{70}). It is difficult to study gene regulation in E. chaffeensis due to lack of transformation system and because the pathogen and its related rickettsials do not naturally harbor plasmids. We developed an E. coli-based transcription system to study E. chaffeensis transcriptional regulation. An E. coli strain with its endogenous σ^{70} gene expression repressed with the trp repressible promoter is used to express E. chaffeensis recombinant σ^{70}. The modified E. coli strain and the previously established in vitro transcription system were then used to map differences in the transcriptional activity of two E. chaffeensis p28 Omp genes, as their gene expression is driven primarily by the E. chaffeensis σ^{70}. We mapped the contributions of -10 and -35 motifs and the AT rich spacer sequences located between these two motifs by performing numerous deletion and point mutation analysis spanning these two gene promoters. The study revealed that the highly conserved -35 motif of these two genes (TTGCTT) is very similar impacting the transcription, while -10 motifs do not contribute to the promoter activities. The variable AT-rich spacer sequences of the two differentially expressed genes contribute to transcriptional differences. We further demonstrated that the protein domains 4.2 and 1.1 of E. chaffeensis σ^{70} interact with the -35 motif and the AT-rich spacer sequence, respectively. This work is the first in defining the molecular basis of differential gene expression assessed at the transcription level of two closely related and differentially expressed E. chaffeensis genes.

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Cerebral nematodiasis in camelids: a retrospective study

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Objectives: To investigate signalment, history, clinical signs, clinicopathological values, CSF cytology, treatments and outcome in camelids diagnosed with cerebral nematodiasis (CN), and to identify variables associated with survival.
Methods: A retrospective study was performed. Camelids (alpacas and llamas) were included if they were diagnosed with CN (based on CSF eosinophilic pleocytosis or consistent postmortem findings). The study period was from 1995 to 2015. Descriptive statistics and logistic regression were performed, with significance set at $P < 0.05$.
Results: Twenty camelids met the inclusion criteria, including 11 alpacas and 9 llamas. The mean age at admission was 5.8 years, and 79% of camelids were male. Common clinical abnormalities at admission included tachypnea (55% of animals), recumbency (55%), ataxia (35%), cranial nerve deficit(s) (25%), and cervical torticollis (20%). Peripheral eosinophilia was not present in any animal, and hyperfibrinogenemia was present in 14% of animals. The most common serum biochemical abnormality was hyperglycemia (60%). The mean percentage of CSF eosinophils was 58%. Of the 85% of treated animals, all received oral fenbendazole, and 88% received a NSAID. The survival rate to discharge was 45%, and there was no association between recumbency at admission and survival ($P = 0.17$). Alpacas were more likely to survive than llamas ($P < 0.01$), females were more likely to survive than males ($P < 0.01$), and hyperfibrinogenemia and treatment with steroids were associated with non-survival ($P = 0.02$ and $P < 0.01$, respectively). Alpacas and females were 21 and 28 times more likely to survive than llamas and males, respectively. These differences were not due to body weight. Steroid treatment increased the risk of death by 49 times.
Conclusions: Male llamas are more likely to die from CN than female llamas or alpacas. Peripheral eosinophilia is absent in animals with CN, despite CSF eosinophilic pleocytosis. Steroid treatment is contraindicated in animals with CN. Although the prognosis for CN in camelids that present to a referral institution is guarded, the presence of recumbency at admission is not predictive of non-survival.

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Molecular detection of vector-borne agents in dogs from ten provinces of China

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Although vector-borne agents are potential zoonoses and cause substantial morbidity and mortality in dogs worldwide, there are limited data on these organisms in dogs of China. Quantitative PCRs for vector-borne agents were performed to investigate their prevalences in convenience whole blood samples obtained from 1,114 dogs from 21 veterinary clinics and a commercial dog breeding facility in ten provinces of China. DNAs of *Babesia gibsoni* and *B. vogeli* (1.2 %), *Ehrlichia canis* (1.3 %), *Hepatozoon canis* (1.8%) and *Theileria orientalis* (0.1 %) were found in the bloods of the dogs studied. Overall, 4.4% (49/1,117) of the dogs studied were positive for at least one vector-borne agent and at least one

vector-borne agent was found in dogs from 5 of the 10 provinces investigated in this study. This is the first report of *T. orientalis* DNA from dog blood specimen.

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Defining the long-term duration of parasitemia and antibody response in cattle infected with various strains and doses of babesia bovis and evaluating sero-diagnostic tools

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Defining the long-term duration of B. bovis parasitemia and antibody response in cattle infected with diverse B. bovis strains and doses is crucial for developing effective control measures for transmission of this important trans-boundary disease. Herein, parasitemia and B. bovis-specific antibody response were defined in calves infected with high and low doses of three B. bovis strains. All six experimentally infected cattle had recurrent parasitemia lasting more than 10 months post-infection even in those infected with a low dose of attenuated B. bovis strains, indicating the persistent infection capacity of all three B. bovis strains regardless of pathogenicity and challenge dose. Low frequency of detectable parasitemia in calves challenged with attenuated strains Mo7 and Tf-137-4 and inconsistent parasitemia in the calf challenged with pathogenic strain T2Bo suggest lack of reliability of parasitemia detection-based diagnosis due to a narrow window of detection, particularly in the carrier stage. In contrast, all six calves maintained robust B. bovis antibody responses during all 12 months of the monitoring period following initial detection at 14 to 15 days post-inoculation when analyzed by a low throughput indirect immunofluorescence assay (IFA). Persistence of B. bovis-specific antibody responses at all tested days after the first appearance, even on days with no detectable parasitemia, indicates the relative advantage of antibody-based diagnostics over antigen detection assays. A previously reported cELISA based on an epitope in rhoptry-associated protein-1 was not reliably antibody positive after 8 months post-inoculation. The diagnostic specificity of the cELISA against negative sera collected in Texas using IFA as the reference assay was 90.4%. Additionally, diagnostic sensitivity of the cELISA was 60% against samples collected in several areas of Mexico against reference IFA. It is posited that development of a high-throughput sero-diagnostic assay with better diagnostic sensitivity/specificity (>98%) against sera from global bovine herds and its use may be pivotal in preventing the spread of B. bovis from endemic to non-endemic areas.

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The epidemiological status of African swine fever in domestic swine herds in the Tavush Marz region, Republic of Armenia.

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African Swine Fever (ASF) is a highly infectious and lethal disease of swine. Introduced in 2007, ASF significantly decreased domestic swine production in Armenia. Most of the 2007 outbreaks were recorded in the Northern areas that border Georgia. The initial onset of the disease was followed by four years of sporadic outbreaks until 2010-2011 when the disease reemerged affecting swine herds in almost the entire country, including cases in feral pigs and wild hogs. No ASF cases have occurred since then. Similar patterns of disease manifestation are observed in some areas of Sub-Saharan Africa where the disease is endemic. Here, historical reports show that ASF outbreaks tend to be sporadic, re-emerging irregularly after intervals of several years without reported cases. In Africa, ASF virus (ASFV) establishes a natural reservoir by cycling between *Ornithodoros* ticks, warthogs and bushpigs. In Armenia, the potential for ASFV infection of indigenous ticks or the continuous transmission of the virus between wild and domestic pigs exist, creating conditions for endemic and epidemic ASF. An active surveillance program was established in Armenia to determine the epidemiological status of ASF focusing on an area at high risk, the Tavush Marz. This Marz was the first to report the presence of ASF in the Armenia in 2007 and 2010-2011. It shares a border with Georgia, where the disease was first detected, that is subject to nearly continuous transboundary movement of people, goods and animals. Most of the pigs in Tavush are bred in small backyard operations and allowed to free-forage, making them more prone to coming in contact with wild pigs and ticks. Samples, including blood, serum and nasal swabs, were obtained from 1500 domestic pigs from 32 communities in the Marz and tested for the presence of ASF by qPCR, ELISA and IPT. Fifty nine ticks were also collected, but the *Ornithodoros* genus was not identified among the collected ticks. All of the samples were negative for ASFV or ASF antibodies suggesting that AFSV is not circulating in the sampled population; however, the question regarding the involvement of wild boars and ticks in ASF epidemiology in Armenia and their role as a reservoir of ASFV remains unanswered.

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Severe Fever with Thrombocytopenia Syndrome virus noncoding regions of S, M and L segments regulate RNA synthesis

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Severe Fever with Thrombocytopenia Syndrome (SFTS) is an emerging hemorrhagic fever that has become a substantial risk to global public health. The causative agent, SFTS virus (SFTSV) is a novel member of the Bunyaviridae family. Ticks are suspected to be the potential vector and SFTSV antibodies were detected in many animal species. In the present study, we analyzed the role of noncoding regions (NCRs) of the three SFTSv RNA segments (L, M, S) in replication, and packaging. We have developed T7 RNA polymerase based mini-genome and packaging systems to investigate the molecular biology of this deadly pathogen in BSL-2 facility. We demonstrated that all necessary signals for RNA encapsidation, and replication are located within these flanking NCRs. Furthermore, comparative analysis showed that replication efficacy varied for different NCRs and was strongest for M followed by L and S segments. Our findings with chimeric minigenomes containing NCRs derived from different SFTS virus RNA segments showed that swapping of NCRs adversely affected the replication levels. Finally, we found that the intergenic region (IGR) located between the two open reading frames (Np and NSs) of the ambisense SFTS virus S segment is critical for optimal

replication. In conclusion, our results show that the sequence elements in the NCRs regulate replication of SFTS viral genomic segments.

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Characterization of the humoral immune responses to porcine epidemic diarrhea Virus (PEDV) infection in weaned pigs

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PEDV causes effusive diarrhea in pigs of all ages and significant mortality among naive pre-weaning pigs. Serology is a tool utilized in control and understanding disease epidemiology. PEDV antibodies have been detected in swine sera by IFA, ELISA and SVN tests. However, detailed evaluation of humoral immune response against PEDV has not been described. The objective of this study was to assess the humoral immune ontogeny in pigs following experimental PEDV infection. Ninety-seven, 3-week-old pigs were allocated into control and challenged groups. Challenged pigs were orogastrically inoculated with 1 ml of 1x103 PFU/ml of PEDV isolate (US/Iowa/18984/2013). The pigs were monitored for clinical signs and fecal shedding of the virus. Serum samples were collected on day 0 and every 7 days till dpi 76 and tested by IFA, ELISA, SVN and Western immunoblot (WIB). IFA was optimized and performed by using virus infected Vero cells and transient expression of PEDV structural proteins in BHK-21 cells. ELISA was optimized for simultaneous detection of IgG, IgM and IgA antibodies against PEDV. WIB was optimized and performed using recombinant structural proteins separately. Antibody response to PEDV was detected as early as 7-14 days. IgA and IgG ELISA antibodies peaked at 21 and 28 dpi respectively and started to gradually decline. Among the viral structural proteins, antibody response against S, and N proteins was detected by IFA as early as 7 dpi followed by M and E proteins. WIB analysis yielded similar results. Overall, pigs developed virus-specific antibody of all major isotypes as early as 7-10 days suggesting serology would be a useful tool to monitor pigs for PEDV exposure. In addition, all major structural proteins of PEDV are immunogenic.

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Porcine reproductive and respiratory syndrome virus hijacks nanotubes for intercellular spread: an alternative pathway used for nidovirus transmission

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The primary mechanism for spreading of most viruses, including porcine reproductive and respiratory syndrome virus (PRRSV) and other nidoviruses, is through the entry of cell-free virions into naïve host cells. In this study, we found an alternative pathway for PRRSV transmission, in which the virus uses the intercellular nanotube connections to spread viral infectious core materials to neighboring cells. In PRRSV infected cells, nanotubes were observed to connect two distant cells with contiguous membranes under the confocal microscopy, and the core infectious viral machinery (genomic RNA and viral proteins) was observed being present inside the intercellular nanotube connection. A live-cell movie of PRRSV containing green fluorescent protein (GFP)-tagged nsp2 shows viral protein moving from one cell to another through a nanotube connection. This phenomenon was also observed in cells infected with equine arteritis virus and porcine epidemic diarrhea virus, suggesting that intercellular nanotube connections serve as an alternative pathway for cell-to-cell spreading of the nidoviruses. In MARC-145 cells expressing PRRSV receptor, infectious viral core materials were still detected moving from one cell to another under the condition of existing viral neutralizing antibody, while in HEK293-T cells lacking PRRSV receptor, intercellular transport of viral materials was also observed in cells transfected with PRRSV genomic RNA. The intercellular nanotube connections contain a core of filamentous actin (F-actin) with myosin associated as a molecular motor. Immunoprecipitation results showed that PRRSV nsp1β, nsp2-related proteins and GP5 can be co-precipitated with F-actin and myosin-IIA. The presence of drugs inhibiting actin polymerization or the myosin-II activation precluded the formation of nanotubes and viral clusters in PRRSV-infected cells. Similar results were also observed with several other nidoviruses. These data lead us to propose that PRRSV (and possibly other nidoviruses) hijacks cytoskeletal machineries for cell-to-cell spread and the intercellular nanotubes could be used as an alternative pathway for the virus to escape the host immune response.

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Mutations in a highly conserved motif of nsp1beta protein attenuate the innate immune suppression function of porcine reproductive and respiratory syndrome virus (PRRSV)

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PRRSV nonstructural protein 1beta (nsp1beta) is a multifunctional viral protein, which involves in suppressing host innate immune response and activating a unique -2/-1 programmed ribosomal frameshifting (PRF) signal for the expression of frameshifting products. In this study, site-directed mutagenesis analysis showed that R128A or R129A mutation introduced in a highly conserved motif (123GKYLQRRLQ131) reduced the ability of nsp1beta to suppress IFN-beta activation and also impaired nsp1beta's function as PRF transactivator. Three recombinant viruses, vR128A, vR129A and vRR129AA, carrying single or double mutations in the GKYLQRRLQ motif were created. In comparison to the wild type (WT) virus, vR128A and vR129A showed similar growth ability, while vRR129AA mutant had reduced growth ability in infected cells. Consistent with the attenuated growth phenotype in vitro, the pigs infected with nsp1beta mutants had lower level of viremia than that of WT virus-infected pigs. In cell culture systems, all of the three mutated viruses stimulated higher level of IFN-alpha expression and exhibited reduced ability in suppressing ISG15 production, in comparison to that of WT virus. In pigs infected with nsp1beta mutants, IFN-alpha production was increased in the lungs during early time points of post-infection, which was correlated with increased production of IFN-gamma in those mutated viruses-infected pigs. These data indicate that PRRSV nsp1beta plays an important role in the modulation of host immune response. Modifying

the key residues on the conserved GKYLQRRLQ motif could attenuate the virus growth and improve PRRSV specific immune responses.

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Attenuation of porcine reproductive and respiratory syndrome virus (PRRSV) by inactivating the ribosomal frameshifting products nsp2TF and nsp2N: Implication for the rational design of vaccines

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PRRSV nonstructural proteins, nsp2TF and nsp2N, were recently identified to be expressed by ribosomal frameshifting during translation of the nsp2-coding region. The nsp2, nsp2TF and nsp2N share the N-terminus PLP2 domain that was previously identified to possess innate immune suppression function. In this study, two recombinant viruses, KO1 and KO2, were further analyzed. KO1 was generated by partial inactivation of nsp2TF expression, while for KO2, the expression of both nsp2TF and nsp2N were knocked out. A multiplexed digital mRNA profiling array assay was employed to detect the expression of 579 immune genes in MARC-145 cells infected with wild-type (WT) virus, KO1 or KO2 mutants. In comparison to that of WT virus, a total of 12 and 88 differentially expressed immune genes were upregulated in cells infected with KO1 and KO2, respectively. KO2 mutant showed significant increased level of expression for certain immune genes (2 to 223-fold increase in expression compared to that caused by WT virus). Major groups of upregulated immune genes involved in cytokine-cytokine receptor interaction, antigen processing and presentation, and TLR, RIG-I and JAK-STAT pathways were identified. Increased levels of mRNA expression for type 1 IFN-a, IFN-b, and IFN-stimulated genes of GBP1, BST2, IFITM1, IFI35, IFIH1, IFIT2 and Mx1 were detected. The upregulation of 15 representative genes was validated by quantitative RT-PCR using samples from WT virus and mutants-infected MARC-145 cells, as well as in lung tissues from WT virus or mutants-infected pigs. Upregulated immune gene expression levels were correlated with increased NK cell activity and T-helper cell response in mutants-infected pigs. Furthermore, vaccination with KO1 and KO2 mutants enhanced homologous protection against a challenge infection, as was evident from reduced lung lesions and viral loads. Our data strongly implicate PRRSV nsp2TF/nsp2N in viral immune evasion and demonstrate that nsp2TF/nsp2N-deficient viruses are capable of generating protective immune responses. Thus, manipulation of nsp2TF/nsp2N expression could be used in the rational design of improved PRRSV vaccines.

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Effects of adenoviral delivered interferon-alpha on porcine reproductive and respiratory syndrome virus infection in swine

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Type I interferons, such as interferon alpha (IFN-α), contribute to innate antiviral immunity by promoting production of antiviral mediators and also play a role in the adaptive immune response. Porcine reproductive and respiratory syndrome (PRRS) is one of the most devastating and costly diseases to the swine industry world-wide and has been shown to induce a meager IFN-α response. We administered porcine IFN-α using a replication-defective adenovirus vector and challenged with a moderately virulent PRRSV. There was a better clinical outcome in pigs treated with IFN-α, including lower febrile responses and decreased percentage of lung involvement. Viremia was delayed and there was a decrease in viral load in the sera of pigs treated with IFN-α. In addition, there was an increase in the number of virus-specific IFN-γ secreting cells, as well as an altered cytokine profile in the lung 14 days post-infection, indicating that the presence of IFN-α at the time of infection can alter innate and adaptive immune responses to PRRSV. In a subsequent experiment we further explored the use of IFN-α as an adjuvant given with attenuated PRRSV virus vaccine and found that it was able to totally abolish replication of the vaccine virus resulting in no seroconversion. Current studies are examining its use as a metaphylactic treatment for an outbreak of PRRSV to both treat and prevent spread of the virus. The use of immunomodulators is a promising area for therapeutic, prophylactic, and metaphylactic use to prevent and combat infectious disease.

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Both CD4+ and CD8+ T cells effectively suppress PRRSV replication in monocyte-derived macrophages

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Porcine reproductive and respiratory syndrome virus (PRRSV) is a difficult-to-control pathogen, due to extraordinarily high mutation and antigenic variation among global isolates, with two distinct genotypes and several subtypes in each genotype. Live attenuated vaccines induce partial protection including reduced plasma viremia and clinical disease before a delayed appearance of weak neutralizing antibody responses in pigs, suggesting a contribution of cell-mediated immunity (CMI). However, efficacy and mechanisms of CMI against diverse PRRSVs need to be defined using a physiologically relevant assay. Herein, a novel 7 day CMI assay was developed using a low dose of PRRSVSD23983, monocyte-derived macrophage (MDM) targets, and T lymphocyte effectors. This assay was used to test the hypothesis that PRRSV-specific cytotoxic T-cells are the protection correlate in PRRSV-infected pigs. Sow 2 PBMCs stimulated for 7 days with PRRSVSD23983 reproducibly suppressed PRRSV replication in 95.5-99.6% of PRRSV-susceptible autologous MDMs and MHC-matched heterologous MDMs. The suppression efficacy of sow 2 PBMCs was insignificantly reduced after depletion of CD8+ T cells; 95-98.3% of PRRSV-susceptible MDMs by CD8-depleted PBMC. Sow 1 and 3 PBMCs suppressed PRRSV replication in autologous MDMs and MHC-matched heterologous MDMs at 83.7-90.1%, but their suppression efficacies were partially reduced to 44.4-60.6% after CD4 or CD8 depletion. Surprisingly, a highly effective suppression by sow 2 effectors was also possible in MDMs from a MHC-mismatched sibling sow, suggesting low stringency MHC restriction in epitope presentations on MDMs with different MHC class-II haplotypes. The data in this study strongly supports that PRRSV-specific CD8+ and CD4+ cells effectively suppress PRRSV replication in PRRSV-infected sows with different haplotypes.

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Both CD4+ and CD8+ T-cells recognize porcine reproductive and respiratory syndrome virus epitopes and lyse infected macrophages in a biphasic mode

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Porcine reproductive and respiratory syndrome virus (PRRSV) is an economically important, difficult-to-control pathogen due to an extraordinary mutation rate, high antigenic variation, and immune modulation in infected pigs. Pigs immunized with live attenuated vaccines induce partial protection without significant neutralizing antibody responses, suggesting potential contribution of cell-mediated immunity (CMI). However, the efficacy and mechanism of CMI responses are still needed to be defined using a physiologically relevant and sensitive assay. We hypothesized that cytotoxic T-cells control PRRSV in pigs. As a tool to test this hypothesis, a cytotoxicity assay (CA) that measures the cytolytic activity of PRRSV-specific T-cells was developed using PRRSVSD23983, monocyte-derived macrophage (MDM) targets, and T-cell effectors. Using the CA, the features of protective cytotoxic T-cells (CTL) were defined as follows. First, T-cells in re-stimulated sow PBMCs reproducibly delivered granzyme-B to PRRSV-infected MDMs. Second, T-cells contributing to cytolysis of PRRSV-infected MDMs had both CD4+ and CD8+. Third, both phenotypes of CTLs could recognize PRRSV epitopes presented from incoming virions before de-novo synthesis of PRRSV proteins after RNA transcription. Fourth, the major contributors to the biphasic cytotoxicity kinetics against PRRSV-infected MDMs were CD4+CD8+high and CD4+CD8- cells in early and late cycles of PRRSV replication, respectively. These results may be valuable for developing an efficacious T cell-focused vaccine which can effectively control infections with diverse PRRSV strains.

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Amino acid residues Ala283 and His421 in the RNA-dependent RNA polymerase of porcine reproductive and respiratory syndrome virus play important roles in viral Ribavirin sensitivity and quasispecies diversity

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The quasispecies diversity of RNA viruses is mainly determined by the fidelity of RNA-dependent RNA polymerase (RdRp) during viral RNA replication. Certain amino acid residues play an important role in determining the fidelity, and such residues can be substituted with other amino acids to produce high fidelity viral strains. In this study, under the selection of Ribavirin two amino acid substitutions (A283T, H421Y) in the RdRp of porcine reproductive and respiratory syndrome virus (PRRSV) were identified. The two substitutions were subsequently found to confer PRRSV the properties of increased Ribavirin-resistance and restricted quasispecies diversity. The results indicated that these two amino acid residues (Ala283 and His421) play a crucial role in PRRSV replication by affecting the fidelity of its RdRp. The results have important implication for understanding the molecular mechanism of PRRSV evolution and pathogenicity, and developing safer PRRSV modified live-attenuated vaccine (MLVs).

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Management Practices Implemented following an outbreak of Porcine Reproductive and Respiratory Syndrome in commercial swine breeding herds in North America

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There is a disconnect between common PRRS outbreak management practices and their known value and demonstrated effect in production following an outbreak of the disease. This lack of evidence, is preventing swine production herds and their veterinarians from developing evidence based effective management practices when a PRRS outbreak occurs. Veterinarians practicing in the US who were members of AASV were asked to report which management practices that they had suggested for the last 5 PRRS outbreaks in herds under their direct management. Questions about management practices were based on the AASV PRRS Task Force recommendations (2012). Preliminary data (N=50 herds, of a target of 250) were analyzed. The goal of the PRRS management plan was evenly distributed between elimination and control of the virus at the time of the outbreak. Conditions and location of the herd (area pig density, flexibility in production numbers, and previous success in stabilizing PRRS transmission and preventing new introductions) were reported as primary reasons to choose the management plan goal. There was a wide variation in practice implementation across herds and veterinarians. “All-in, All-out” management, washing, disinfecting and drying of all farrowing crates/pens between litters is was common across herds in the immediate post break period regardless of herd goal. Methods for monitoring and sampling the herd to determine herd status showed consistency with respect to numbers and sampling of due to wean pigs with common use of PRRS ORF5 rtPCR testing. In phase 2 of the project we will compare production outcomes (total piglet losses from baseline) and the sub metrics that determine total herd productivity between herds with high and low levels of PRRS specific management practices that were implemented. Management practice data from all herds in the survey will be presented.

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Attenuation of US original porcine epidemic diarrhea virus strain PC22A via continuous cell culture passages

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Purpose:

The challenge of continuous porcine epidemic diarrhea (PED) outbreaks caused by the emerging PED virus (PEDV) in the US necessitates the development of effective vaccines. Attenuated PEDV generated via serial cell culture passages can serve as live attenuated vaccine candidates.
Methods:
The original US highly virulent PEDV strain, PC22A, was continuously passaged in two different Vero cell lines (CCL81 and BI cells). The virulence of passage level 95 [P95 (CCL81)], P100 (BI) and P120 (BI) was assessed in 4-day-old, cesarean-derived, colostrum-deprived (CDCD) piglets by orally inoculating piglets with 100 plaque forming units (PFU) per pig. Virulent PC22A-P4 and mock-inoculated piglets were used as positive and negative controls, respectively.
Results:
From ~P65 (BI), the viral infectious titers increased 3 $\log_{10}$ to titers of 8 $\log_{10}$ PFU/ml. Sequence analysis of the spike (S) gene showed that P95 (CCL81) has one amino acid (aa) insertion and five aa substitutions, and P100 (BI) had one aa insertion, two aa deletions and eight aa substitutions compared to PC22A-P4. Additional one aa substitution and nine aa-deletion at the end of the S protein were identified in P120 (BI) compared to P100 (BI). Further full genomic sequence analysis is ongoing. Both PC22A-P4 and P95 (CCL81) caused severe diarrhea and mortality in 100% of CDCD piglets. P100 (BI) and P120 (BI) had much decreased virulence based on delayed virus shedding, decreased and delayed onset of clinical signs, milder intestinal lesions in histopathology and lower viral antigen scores. High dose (5 $\log_{10}$ PFU/piglet) infection of P120 (BI) in conventional suckling piglets will be performed to verify whether P120 is attenuated.
Conclusion:
Our results demonstrated that different Vero cell lines might provide various selection pressures on PEDV replication *in vitro*. Mutations accumulated through passages resulted in higher virus titers *in vitro* and decreased virulence *in vivo*.

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Comparison of porcine epidemic diarrhea virus (PEDV), transmissible gastroenteritis virus (TGEV) and porcine deltacoronavirus (PDCoV) for pathogenicity in weaned pigs

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TGEV has been a major enteric pathogen of US swine since 1946. The emergence of PEDV and PDCoV in US swine created awareness to their economic impact. While pathogenicity of these individual enteric coronaviruses in pigs has already been documented, there is no data comparing pathogenicity of each swine virus against each other. Therefore, we evaluated the pathogenicity of these three swine coronaviruses simultaneously. One hundred 8 week old pigs were allotted to one of the following treatment groups: (n = 25 pigs/group) included: 1) Control; 2) PEDV inoculated; 3) TGEV inoculated; and 4) PDCoV inoculated. At 0 days post inoculation (dpi), all pigs received a gastric gavage of their designated viruses (10^3 $TCID_{50}$/ml) or a sham inoculum for the control group. Serum, feces, and oral fluids were sampled from each pig daily during the first 5 dpi. Subsequently, weekly sampling occurred. In addition, body weight and feed intake were recorded weekly to monitor growth performance of the pigs per treatment group. All infection treatment groups were detected positive by quantitative RT-PCR. After 2 dpi, differences were observed in the virulence and viral shedding between the treatment groups. PDCoV and TGEV infected pigs shed virus in the feces for up to 3 dpi, while PEDV was detectible in feces for up to 7 dpi. PEDV infected pigs had the greatest reduction in growth performance based on ADG and ADFI. In gastrointestinal tract, PEDV RNA was most persistently present in both the small and large intestinal tissues with the highest viral load when compared to TGEV and PDCoV groups. Overall, the pigs infected with PEDV had more severe clinical manifestations with the greatest viral load, while the TGEV and PDCoV infections were less severe under the conditions of this investigation.

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Prevalence of multi-drug resistance in E. coli and enterococci organisms isolated from abattoir workers and broilers

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This study investigated prevalence of Multi-drug resistance in broilers raised on antimicrobial feed additives, and abattoir and non-abattoir workers. It also assessed predictors of MDR.
Broiler caeca (n=100) randomly sampled at an abattoir and human faecal samples were selectively cultured to isolate *E. coli* and enterococci. Minimum inhibitory concentrations were determined for several antimicrobials. Prevalence of MDR was computed and predictors of MDR were investigated using multivariable logistic regression.
E. coli from poultry exhibited higher prevalence of MDR (94.4%) compared to abattoir workers (75%) and non-abattoir workers (42%). The majority of poultry MDR *E.coli* isolates were resistant to: doxycycline (98.2%), nalidixic acid (87.4%), sulphamethoxazole (79%), enrofloxacin (76.1%), and erythromycin (76.1%). MDR enterococci from poultry were mainly resistant to: doxycycline (100%), sulphamethoxazole (73.7%), bacitracin (63.2%), enrofloxacin (57.9%), erythromycin (100%), fosfomycin (100%), and ceftriaxone (84.2%). The odds of human *E. coli* isolates being MDR (OR = 0.03) was lower than for *enterococci*. Significant predictors of MDR in human isolates was resistance to doxycycline (OR=8), fosfomycin (OR=16) and sulphamethoxazole (OR=20) whereas only doxycycline (OR=46) and *E. coli* (OR = 1.5) were significant in poultry isolates. Moreover, there was no evidence that abattoir work affected risk of MDR.
Monitoring antimicrobial resistance, and efforts to minimizing selection pressure responsible for the high prevalence is needed. Since MDR among human isolates was more common in enterococci than *E. coli* isolates, enterococci may be better indicators of resistance in humans. While resistance to doxycycline, fosfomycin, and
sulphamethoxazole were identified as predicators of MDR in human isolates, only doxycycline was a predicator of MDR in the poultry. Authors could not conclude if working at the abattoir was significantly associated with MDR.
Key words: Predictors; multi-drug resistance; *E. coli*; Enterococcus spp.; risk factors; abattoir workers; broilers; poultry.

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A computationally designed indirect ELISA for the detection of porcine epidemic diarrhea virus (PEDV) - specific antibodies

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Porcine epidemic diarrhea virus (PEDV) causes acute and severe diarrhea and vomiting in pigs of all ages; affecting 100% of exposed animals and causing mortality rates of up to 50%. The emergence and rapid spread of PEDV in 2013 in the U.S resulted in severe economic devastation and losses, underscoring the need for improved technology for the development of rapid-response diagnostics and vaccines for newly emerging infections. In this study, we used computational methods to predict the antigenicity of selected regions of the N and S proteins. Two diagnostic targets were designed based on the analysis. The targets were synthesized in a rapid and inexpensive manner using an in-vitro transcription and translation kit. Their performance in an ELISA was compared to a traditional assay using bacterially expressed PEDV nucleocapsid (N) protein and an indirect immuno-fluorescence assay (IFA). Using the IFA as the gold standard, ROC analysis of data from 173 samples tested showed that the sensitivity and specificity of the conventional N protein ELISA was 86.3% and 92.93% at a cut-off value of 0.389. When compared to the IFA and conventional N protein ELISA, the sensitivities of the computationally designed N and S protein ELISA's were 95.92% and 93.88% respectively, while the specificities were 86.27% and 94.12% respectively. This study provides proof-of-concept for the design of rapid response immuno-assays in outbreak situations.

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Does systemic antibody play a role in the protection of piglets against PEDV?

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Many questions remain regarding the role of maternal anti-PEDV antibody in the protection of neonates against PEDV. This experiment focused on the impact of maternal (colostral) antibody on the course of PEDV replication and neonatal health using "passive transfer model".

METHODS

6 PEDV IFA-negative sows were acquired from commercial swine farms at ~110 days of gestation. After farrowing, piglets (n=62; 2-to-3 days of age) were intraperitoneally (IP) administered 1 of 6 levels of concentrated PEDV antibody sufficient to achieve circulating FFN antibody titers of (<1:8, 1:5.3, 1:6.1, 1:8, 1:17.1, and 1:32). 24 h later, piglets were inoculated with PEDV and observed through 14 days. Piglets remained on the dam throughout the study.

Clinical outcomes, sow milk, piglet fecal samples, body weight, and body temperature were collected on daily basis and serum samples were collected from piglets at DPI -1, 0, and 14, or at the time of humane euthanasia. Fecal samples were tested by PEDV rRT-PCR. Piglet serum samples were tested for PEDV IgG and IgA (ELISA) and for PEDV FFN antibody. The effects of treatment on the outcomes measured were analyzed using repeated measures ANOVA.

RESULTS

PEDV circulating antibody had no effect on piglet growth and duration or viral concentration of PEDV shedding in feces.

The presence of antibody modified the body temperature response in infected piglets. The treated piglets recovered from hypothermia by DPI 4, whereas negative control piglets recovered on DPI 7.

Negative control pigs had the lowest survival rate (9.1% vs 20-50% in treated groups).

The presence of circulating anti-PEDV antibody suppressed the humoral response of inoculated piglets, i.e., piglets that received passive antibody had significantly lower levels of antibody at 14 DPI as measured by FFN, PEDV IgA ELISA, and PEDV IgG ELISA tests.

CONCLUSION

The results suggested that circulating antibody can alter some parameters of PEDV infection in neonates (body temperature response, increased survivability, inhibited the humoral response), but not others (piglets' growth, duration of PEDV fecal shedding). These results have implications for the management of PEDV in commercial herds.

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Transduction of hematopoietic stem cells to stimulate RNA interference for treatment of feline infectious peritonitis

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Feline infectious peritonitis (FIP) is a highly fatal disease caused by virulent feline coronavirus (FCoV) that has the ability to infect monocytes/macrophages. Lack of an effective host immune response against the virus results in systemic virus proliferation. In a previous study, we proved that RNA interference (RNAi) can be used to inhibit FCoV replication in vitro. RNAi, mediated by small interfering RNA (siRNA), has therapeutic potential if the siRNA can be delivered in sufficient quantity to monocytes/macrophages. The goal of the current study is to assess the feasibility of transducing hematopoietic stem cells (HSCs) with FCoV-specific, siRNA-coding DNA (miRNA) by ex vivo introduction of a non-replicating lentivirus vector.

To assess the effectiveness of the designed miRNAs to inhibit viral replication, stably transduced CrFK cells were initially prepared and infected with FCoV. Inhibition of coronavirus replication was determined by qReal-time RT-PCR and TCID 50 assay. Three miRNAs; microRNA-L1, L2 and microRNA-N that targeted the leader sequence and the nucleocapsid gene, respectively exhibited variable inhibitory effects on viral replication in vitro. miRNA-L1 and miRNA-N resulted in less than 50% reduction in FCoV genomic RNA (gRNA) synthesis as compared with the negative control sample. miRNA-L2 resulted in 64% and 73% reductions in FIPV WSU 79-1146 and FECV WSU 79-1683 gRNA synthesis, respectively as compared with the negative control sample. HSCs have been obtained from feline bone marrow and replicated in vitro. Stably

transduced HSCs that express miRNA-L2 were produced and evaluated for toxicity and ability to inhibit FCoV replication in vitro. miRNA-L2 resulted in 90% and 92 % reductions in FIPV WSU 79-1146 gRNA synthesis and extracellular virus production respectively, as well as 74% and 80% reduction in FECV WSU 79-1683 gRNA synthesis and extracellular virus Production. These preliminary results suggest that genetic modification of HSCs for constitutive production of these anti-coronavirus siRNA will reduce FCoV replication. This proof of concept work will potentially lead to in vivo introduction of these genetically modified HSCs for the treatment of FIP.

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Development of a snatch farrowed-colostrum deprived piglet challenge model for porcine Rotavirus C

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Porcine rotavirus C (PRoV) is an environmentally stable pathogen in piglets. Culture methods for PRoV do not exist. Impeding evaluation of interventions under controlled conditions. We describe a novel snatch farrowed-colostrum deprived (SF-CD) neonatal piglet challenge model for PRoV. Our objective was to determine if PRoV infected animals could be differentiated from control animals by presence of diarrhea, weight gain, and morphological changes in the gastrointestinal (GI) tract. Two milk replacers were evaluated along with PRoV challenge. Sixteen piglets were placed into four treatment groups: Control pigs that remained on the sow until end of experiment (CS), Non-challenged SF-CD pigs on porcine milk replacer (CR), Non-challenged SF-CD on human milk replacer (CH), and rotavirus-challenged SF-CD pigs on porcine milk replacer (RR). On Day 0 (24h of age), rotavirus-challenged pigs (RR) were inoculated with an intestinal homogenate containing PRoV. One pig from CH and CS groups were euthanized prior to enrollment; N=3 for CH and CR treatments, N=4 for RR treatment. All pigs were housed individually, fed via bottle every two hours, and observed for diarrhea by a blinded observer every 24 hours. At 72h post-challenge, piglets were euthanized and samples were collected. 5/10 SF-CD pigs enrolled were removed from study prior to D3; CH=3, CR=0, RR=2 due to sepsis or bronchopneumonia. No CR pigs exhibited diarrhea and 2/4 RR pigs had diarrhea only on D1. CR pigs gained significantly more weight compared to RR pigs from D0-D3 (390g vs. 70 kg, p=0.02). These data suggest both challenge process and infection control procedures were effective. To improve the model more pigs need to be included to account for early neonatal losses and methods to control bacterial disease need to be implemented including use of systemic antibiotics. To further refine the model, dose titration experiments will be conducted to calculate the Pig Infectious Dose 50 (PID50) based on the Karber Equation. In this model we can reliably induce disease due to PRoV that is discernable from expected developmental challenges in SF-CD pigs. We conclude that this model is suitable for assessing efficacy of interventions against PRoV.

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Evaluation of clinical and immune responses following infection of horses with EHV-1 wild type and different EHV-1 mutants

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Equine herpesvirus type 1 (EHV-1) is the cause of a devastating myeloencephalopathy (EHM) in horses worldwide. Despite the importance of EHM, our understanding of its pathogenesis beyond the essential role of viremia is rudimentary. To address questions regarding viral and host factors for the pathogenesis of EHM, the focus of this study was to compare viruses with differing neuropathogenic potential.

Four groups were established: uninfected controls, wild type infected horses (Ab4), polymerase infected horses (Ab4 N752) and gD mutant infected horses (EHV-1 gD4). Animals were assessed for 21 days post infection (p.i.). Blood and nasal swabs were collected prior to infection and for 10 days p.i. and viremia and nasal shedding were analyzed by real time PCR. Nasal secretions and cerebrospinal fluid (CSF) were collected prior to infection and on days 1 & 2 p.i. (nasal secretions) or 11 (CSF) for cytokine measurement. Serum neutralization (SN) titers were determined at regular intervals.

Ab4 N752 infected horses showed most severe EHV-1 respiratory disease followed by Ab4 infected horses. EHV-1 gD4 infected horses showed only mild respiratory disease. Three Ab4 infected horses also developed signs of EHM. Interestingly, only Ab4 infected horses presented a classical bi-phasic fever. Ab4 N752 infected horses showed only primary fevers and EHV-1 gD4 infected horses showed mostly late secondary fevers. EHV-1 SN increased in all groups p.i.. IFN-gamma was down modulated and IL-10 production was completely inhibited in the CSF after Ab4 infection, while no significant changes were observed in the other groups. In contrast with CSF, IFN-gamma was significantly increased in nasal secretions of Ab4 infected horses. Finally, IFN-alpha was not detectable in nasal secretions before infection, but significantly induced following infection in all infection groups.

In conclusion, we detected differences in respiratory and neurological disease of horses infected with EHV-1 viruses of differing neuropathogenic potential. These clinical differences appear to be associated with differing ability of WT and mutant viruses to modulate host responses and induction of Interferons and IL-10.

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Development of a real-time PCR assay for the detection and quantification of equine herpesvirus 5

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Purpose:

Equine herpesvirus 5 (EHV-5) infection has recently been associated with equine multinodular pulmonary fibrosis in horses. To more completely understand EHV-5 pathogenesis, as well as viral and host contributions, further *in vivo* and *in vitro* studies are needed. However, current measurement techniques are unable to rapidly, specifically, and quantitatively characterize EHV-5 infection. The aim of our project was to develop a sensitive and specific TaqMan real-time PCR assay to quantify EHV-5 in clinical and cell culture samples, and use this test to describe viral replication over time in primary equine respiratory epithelial cells (EREC)s.

Methods:

Primers and a probe were designed to target gene E11 of EHV-5 for TaqMan real-time PCR. Specificity was verified by testing multiple isolates

of EHV-5, as well as DNA from other equine herpesviruses. A plasmid containing the target DNA was generated to create a standard curve and quantify viral copy number. TaqMan real-time PCR was performed on DNA isolated from clinical samples. ERECs were inoculated with EHV-5, and then the cells and supernatants were collected daily for 12 days. After isolating DNA from these samples, TaqMan real-time PCR was performed and compared to results obtained by conventional PCR.

Results:

Our TaqMan real-time PCR assay for EHV-5 detected and quantified multiple currently circulating isolates of EHV-5. It did not detect DNA from other equine herpesviruses, including equine herpesvirus 2 - a close homolog of EHV-5. Using TaqMan real-time PCR, we were able to quantify viral copy number in clinical samples, as well as in EHV-5 inoculated ERECs. Quantification was not possible using conventional PCR. Using data obtained from the EREC culture, we established a 12 day viral growth curve in cells from 3 horses.

Conclusions:

In conclusion, this assay is a rapid, specific, and sensitive test that can be used to more accurately describe initial events following EHV-5 inoculation than is possible with previously established techniques. Furthermore, this assay will be useful in future *in vivo* investigations to study the epidemiology and pathogenesis of EHV-5 in its host.

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Sites of equine arteritis virus persistence in the stallion's reproductive tract and characterization of the local inflammatory response to the virus

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Equine arteritis virus (EAV) can establish persistent infection in sexually mature colts and stallions following natural infection. Carrier stallions continuously shed the virus in their semen, and are considered the natural reservoir of EAV. However, the tissue and cellular tropism of EAV in the male reproductive tract and the mechanism(s) that enables persistent infection remain to be elucidated. The primary objective of this study was to identify the tissue localization and EAV cellular tropism in the reproductive tract of experimentally infected, long-term carrier stallions. Samples from the entire reproductive tract were processed for histopathology, detection of viral antigen and cell specific surface markers by single and dual color immunohistochemical (IHC) and immunofluorescent antibody (IFA) staining techniques, *in situ* hybridization (RNAscope®), and electron microscopy (TEM). Histological lesions were characterized by minimal to mild, focal to multifocal lymphoplasmacytic inflammation with occasional perivascular cuffs distributed throughout the reproductive tract. These inflammatory infiltrates comprised $CD2^+$ (low to high), $CD3^+$ (moderate to high), $CD4^+$ (low to moderate), $CD5^+$ (moderate to high), $CD8^+$ (moderate to high) T lymphocytes, $CD25^+$ T regulatory lymphocytes (low to moderate), $CD21^+$ B lymphocytes (minimal to moderate), and $CD83^+$ dendritic cells (low to moderate). Interestingly, viral particles were identified within intracytoplasmic vesicles in fibroblasts and lymphoplasmacytic infiltrates by TEM. This finding is supported by dual IHC/IFA showing viral antigen localized in fibroblasts, lymphocytes ($CD2^+$, $CD3^+$, $CD5^+$, and $CD8^+$ T lymphocytes and $CD21^+$ B lymphocytes), and other mononuclear cells predominantly within the ampulla. Some infected cells were detectable in other accessory sex glands. This study not only unequivocally confirms the ampulla as the primary site of EAV persistence, but also demonstrates that the host cells involved are from fibroblastic and mononuclear lineages, including T and B lymphocytes associated with inflammatory responses rather than glandular epithelia.

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Equine arteritis virus uses equine CXCL16 (EqCXCL16) as a cell entry receptor

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Equine arteritis virus (EAV) is the causative agent of equine viral arteritis (EVA), a respiratory, systemic and reproductive disease of equids. Similar to other arteriviruses, EAV primarily targets cells of the monocyte/macrophage lineage which are believed to play a critical role in EVA pathogenesis. Previous studies in our laboratory have identified equine CXCL16 (EqCXCL16) as a candidate molecule and possible cell entry receptor for EAV. In horses, the CXCL16 gene is located on equine chromosome 11 (ECA11) and encodes a glycosylated, type I transmembrane protein with 247 amino acids. Thus, the primary objective of this study was to confirm that EqCXCL16 is one of the putative receptors for EAV entry into susceptible cells. The study findings demonstrated subpopulations of equine CD14+ monocytes that express EqCXCL16 are preferentially infected with EAV and that infection rates in these cells are reduced by pretreatment with guinea pig polyclonal antisera against EqCXCL16. Moreover, stable transfection of HEK-293T cells with plasmid DNA encoding EqCXCL16 (HEK-EqCXCL16) increased their permissiveness to EAV infection from a very small percentage (<3%) to almost 100% of the cell population. The increase in permissiveness was blocked either by transfection of HEK-EqCXCL16 cells with siRNAs directed against EqCXCL16 or by pre-treatment with guinea pig polyclonal antiserum against EqCXCL16 protein. Furthermore, using a virus overlay protein-binding assay (VOPBA) in combination with Far-Western blotting, gradient purified EAV particles were shown to bind directly to the EqCXCL16 protein in vitro. Finally, binding of biotinylated EAV virulent Bucyrus strain at 4 °C was significantly higher in HEK-EqCXCL16 cells compared to non-transfected HEK cells. The collective data from this study provide confirmatory evidence that the transmembrane form of EqCXCL16 likely plays a major role in EAV host-cell entry processes, possibly acting as a primary receptor molecule for this virus.

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Application of a broad-spectrum microbial detection array for the analysis of pig pathogens

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Many of the disease syndromes challenging the commercial swine industry involve the analysis of complex problems caused by polymicrobial, emerging/re-emerging and transboundary pathogens. Currently circulating transboundary diseases, such as African swine fever and classical swine fever are constant threats and raise concern about the possibility of both intentional as well an unintentional introduction. The best assurance of timely identification for known and unknown threats is to employ techniques that can track known disease threats, as well as rapidly identify the introduction of new pathogens before they become established.
In this study, we employed a novel and comprehensive microbial detection technology, the Lawrence Livermore Microbial Detection Array (LLMDA) that was designed to detect >8000 species of microbes. We evaluated the utility of LLMDA to analyze the microbial composition in serum, oral fluid and tonsil samples from pigs co-infected with porcine reproductive and respiratory syndrome virus (PRRSV) and porcine circovirus type 2 (PCV2).
Under conditions of experimental infection, the array identified PRRSV and PCV2, but at a lower sensitivity compared to PCR. The pen-based oral fluid sample was the most informative, possessing signatures from several porcine-associated viruses and bacteria. In addition to PCV2 and PRRSV, the LLMDA also detected other viral co-infections including porcine parainfluenza, astrovirus, and porcine stool-associated virus from oral fluid samples. Common bacterial co-infections detected by LLMDA are Streptococcus suis, clostridium sp., Staphylococcus sp., and Enterococcus sp.
This study demonstrates the utility of LLMDA in routine clinical diagnostics and surveillance. Even though microarrays are not as sensitive as standard PCR assays, they create the opportunity to query hundreds of thousands to several million sequence-specific DNA signatures, all in parallel. LLMDA is a valuable tool in the analysis of syndromes that result from polymicrobial interactions, and the detection of emerging and transboundary pathogens.

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Detection of antibody responses to the porcine circovirus strain 2 (PCV2) replicase protein.

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Porcine circovirus strain 2 (PCV2) is an important pathogen and the etiological agent of post-weaning multi-systemic wasting disease syndrome, an economically important disease of pigs. Typically, viral non-structural proteins are expressed abundantly in early infection and prior to the expression of structural proteins. Little is known about antibody responses to the major non-structural, replicase protein of PCV2. To understand the kinetics of the antibody responses to the replicase protein, the replicase gene was cloned and expressed using an *in-vitro* transcription translation system. Weekly serum collections from animals that were either infected with PCV2 (N=36) or uninfected (N=12) were tested for the presence of antibodies against the replicase protein by an ELISA. Replicase-specific antibodies were detected as early as the seventh day post-infection while the response had matured by the 14th day post-infection (DPI). In most seropositive animals titers continued to increase until DPI 28. Our preliminary results indicate that the replicase protein is immunogenic and can be used as a target to detect infected animals. As all current PCV2 vaccines are either inactivated or subunit vaccines which contain the capsid protein, vaccinated but uninfected animals should not have antibodies against the non-structural proteins. Therefore, our findings have potential implications for the development of a strategy which can allow for the early detection of infected animals in vaccinated herds.

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Discovery of a novel putative atypical porcine pestivirus in pigs in the United States

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Pestiviruses are some of the most significant pathogens affecting ruminants and swine. Here, we assembled a 11,276 base pair contig encoding a predicted 3,635 amino acid polyprotein from porcine serum with 68% pairwise identity to a recently partially characterized *Rhinolophus affinis* pestivirus (RaPV) and approximately 25-28% pairwise identity to other pestiviruses. The virus was provisionally named atypical porcine pestivirus (APPV). Metagenomic sequencing of 182 serum samples identified four additional APPV positive samples. Positive samples originated from five states and enzyme-linked immunosorbent assays using recombinant APPV Erns found cross reactive antibodies in 94% of a collection of porcine serum samples,suggesting widespread distribution of APPV in the U.S. swine herd. The molecular and serological results suggest that APPV is a novel, highly divergent porcine pestivirus widely distributed in U.S. pigs. Tissues were submitted from a swine farm with large numbers of pigs exhibiting incoordination and tremors over an approximate 10 week period. Mortality of affected pigs approached 100% within 4 days of clinical symptoms. Metagenomic sequencing of brain tissue solely identified APPV which was subsequently confirmed by qRT-PCR and immunohistochemistry. In addition to the brain, APPV was identified in serum, lymph node and spleen. While APPV has not been established as etiological agent of neurological disease of swine, further investigation is warranted.

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Identification of novel Senecavirus A from pigs with vesicular disease in the US

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Senecavirus A (SV-A), formerly known as Seneca Valley virus, is a member of the family Picornaviridae. The virus was initially found as a contaminant of the PER.C6 cell line. Since its discovery, the virus has been detected in pigs with vesicular disease which grossly resembles FMD, swine vesicular disease, vesicular exanthema of swine and vesicular stomatitis. However, clinical significance of SV-A is unclear since disease

has not been reproduced in pigs by experimental infection. In July of 2015 Iowa State University Veterinary Diagnostic Laboratory (ISUVDL) received 4 cases of vesicular disease affecting show and commercial pigs. Animals of the first 3 cases were originated in three unrelated farms in Southwest and Central Iowa and observed at two county fair exhibitions. The fourth case was observed in a commercial finisher farm in the Midwest US which was unrelated to any of the previous 3 cases. Clinically affected animals showed acute lameness, anorexia and pyrexia. Grossly affected pigs exhibited coronary band hyperemia with vesicle formation which was progressed to cutaneous ulcers. Small vesicles were also evident on snout and/or in the oral cavity which were progressed to ulceration. All cases tested negative for vesicular diseases mentioned at USDA's FADDL. No other common swine pathogens except SV-A were detected by PCR assays. Virus isolation attempts using various cell lines yielded SV-A isolate from each case. Sequencing of the isolates for VP1 gene and full-length genomic RNA revealed that the viruses shared 99-100% identity with each other but significantly divergent from historical SV-A isolates (1988-2001) whose sequences are available in GenBank, suggesting that SV-A may have been evolved which may lead to change in its pathogenicity in pigs.

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Protective properties of live and inactivated vaccine based on rescued recombinant influenza A virus against highly pathogenic H5N1 strain in chickens.

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Purpose. The highly pathogenic (HP) avian influenza viruses (AIV) represent a serious economic threat to the poultry industry globally. The viruses of avian origin H5N1 and H7N9 can infect humans following contact with live poultry usually with a low level of morbidity but high death rates among the infected individuals. Vaccinating the livestock can prevent the disease as well as reduce circulation and shedding of AIV, thus protecting the farm workers. The purpose of the present study was to evaluate the efficiency of new re-assortant vaccines that had hemagglutinin (HA) antigens matching the ones from natural infection. Methods. Inactivated emulsified vaccines and live attenuated vaccines were developed on the basis of the following candidate strains that were engineered using reverse genetics: recPR8-H5N1 and recPR8-H7N1 containing the HA genes from HP AIV strains A/Kurgan/05/2005 (H5N1), and A/Anhui/1/2013 (H7N9), the neuraminidase and internal genes from A/Puerto Rico/8/34 (H1N1). The resulting vaccines were used to immunize groups of 6-week-old chickens followed by challenge with AIV strains with a homologous HA on day 14 after immunization. Level of protection from avian influenza, seroconversion, accumulation of the virus in tissues and shedding of the viruses in feces was analyzed. Results. Infection of naive birds with vaccine candidate strains recPR8-H5N1and recPR8-H7N1 did not cause any pathology, and the viruses were not detected by PCR in kidney, spleen, lungs, pancreas or cloacal swabs. Inactivated and live vaccines on the basis of the recPR8-H5N1 strain protected chickens from lethal challenge with HPAIV A/Kurgan/05/2005 (H5N1). Naive birds in control groups died of HPAIV infection within 3 days, and the virus was found to be present in all organs and feces. In sharp contrast, tissues or fecal swabs from chickens that were immunized before exposure did not contain wild type AIVs 2 days after challenge. Conclusions. It was concluded that the new recombinant vaccine strains were not pathogenic, both inactivated and live vaccines prevented the birds from the disease and virus shedding.

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Current understanding on intercontinental HPAI: To vaccinate or not?

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Intercontinental H5 highly pathogenic avian influenza (HPAI) has decimated the poultry industry throughout the Midwestern US and continues to threaten the nation's food supply and the livelihood of poultry related businesses. The unique H5 HPAI virus most likely originated from China and very quickly became fatal. It continues to evolve and we still do not know much about its enormous capacity to change, reassort and spread to different species. In addition to basic research, innovative methods are needed for effective and humane euthanasia of the affected flocks and safe disposal of infected carcasses and contaminated manure to prevent the spread of the virus. Stamping-out remains the primary control and eradication strategy for HPAI in poultry. However, the use of vaccines and vaccination as part of a control strategy for HPAI continues to be a hot topic for the debate. Our current knowledge on pathobiology of intercontinental HPAI, potential control measures including vaccination, and related issues will be discussed.

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Mouse model for the Rift Valley Fever virus MP12 strain infection

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Rift Valley fever virus (RVFV) is the causative agent of Rift Valley fever (RVF) and classified as a Category A pathogen and select agent. To date there is no commercial, fully licensed vaccine available in the U.S. for human or animal use and effective antiviral drugs have not been identified. The RVFV MP12 strain is a vaccine strain commonly used in laboratories that is categorized as a BSL-2 pathogen. However, it is not virulent for mice. In order to evaluate antivirals or vaccines in a BSL-2 facility, it is crucial to develop small animal models that are susceptible to MP12 strain infection. Herein, we investigated susceptibility of six mouse strains (129S6/SvEv, STAT-1 KO, 129S1/SvlmJ, C57BL/6J, NZW/LacJ, BALB/c) to the MP12 virus via an intranasal route of inoculation with an infectious dose of 1.58 ×10*6 PFU/mouse. There was severe weight loss and obvious neurological clinical signs with 50% mortality in the STAT-1 KO mice, whereas inoculation of the other 5 strains of mice did not result in obvious weight loss. Interestingly, there was neurological disease observed at the end of the study (14 days post infection, dpi) in two BALB/c mice. Furthermore, virus replication was detected in brains and livers of the STAT-1 KO mice on 3 dpi and 6 dpi. Histopathological lesions were also found in livers and/or brains of the MP12 infected STAT-1 KO mice that were euthanized on 3 dpi and those

euthanized due to severe clinical disease. Taken together, the STAT-1 KO mouse strain is susceptible to MP12 virus infection and develops disease. The STAT-1 KO model for the MP12 has potential to be used to investigate vaccine and antivirals in a BSL-2 environment.

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Identification and serotyping of Foot and Mouth Disease virus prevalent in Savar upazila of Bangladesh using one-step RT-PCR and multiplex RT-PCR

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Identification and serotyping of Foot and Mouth Disease virus prevalent in Savar upazila of Bangladesh using one-step RT-PCR and multiplex RT-PCR

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Background: Bangladesh is a small country (147570 Km^2) of South Asia with a high density of human population (1015/Km^2) as well as animal population. Most of the people (80%) depend on agriculture, mainly on livestock rearing for food and draught power. But there are many diseases which hinder the production of livestock. Among these Foot and Mouth disease (FMD) is the most detrimental, mainly to cattle. **Objective:** This study aimed to identify the Foot and Mouth Disease virus from suspected cattle and to confirm the serotypes involved in the disease prevalence in the study area. **Methodology:** A total of fifteen clinical samples were collected from live FMD infected cattle showing signs and lesions of Foot and Mouth Disease from different outbreak areas of Savar upazila of Dhaka district of Bangladesh. Firstly simplex one-step RT-PCR was performed using universal primer pair 1F and 1R to identify whether the virus belonged to FMD group or not. After initial confirmation of FMD virus, multiplex RT-PCR (mRT-PCR) was employed using serotype specific primers (P38:P40:P74-77:P110) to confirm the FMD virus serotypes. **Result:** By simplex one-step RT-PCR with universal primer set, thirteen samples (86.67%) were found to be positive for FMDV and amplified the targeted amplicon of 328 bp size. By multiplex RT-PCR, out of the 13 FMD positive samples; 5 samples (38.46%) were found to be positive for O type, 4 samples (30.78%) were positive for A type, and 2 samples (15.38%) were positive for Asia-1 type. There were 2 cases (15.38%) of mixed infection with synchronized presence of A and Asia-1 serotypes. No isolates were positive for FMD C type. **Conclusion:** The study revealed that three FMDV serotypes, O, A and Asia-1 are circulating in Savar upazila (sub-district) of Dhaka district during the study period.

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2015 North American PRRS Symposium Abstracts

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1

Comparison of five commercial ELISA kits for the detection of antibodies against porcine reproductive and respiratory virus (PRRSV) in field serum samples

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The aim of this study was to assess the sensitivity and specificity of five commercial PRRSV ELISA kits available in Europe. The following kits were used: BioChek PRRS ELISA (BioChek, the Netherlands), IDEXX PRRS X3 Ab Test (IDEXX, USA), and INgezim PRRS 2.0 (Ingenasa, Spain), VetExpert PRRS Ab ELISA 4.0 (Bionote, Korea), PrioCHECK PRRSV Antibody ELISA (ThermoFisher, USA). The study was performed on samples obtained from farms with different PRRSV status. Farm I was stable regarding PRRSV. Replacement gilts were vaccinated with Porcilis® PRRS (MSD) modified live vaccine. In Farm II, replacement gilts, as well as 3 weeks old piglets were vaccinated with Porcilis®. In Farm III no vaccination program was applied. In farms I and II, 3 pens, and in farm III 4 pens were selected, and blood samples were collected at 5, 9, 13 and 17 weeks of age, from 4 pigs from each pen. In Farm II and III PRRSV Type 1 was detected. For the specificity analysis 140 sow sera from several, PRRSV naïve farms are being used. In Farm I only maternal antibodies were detected. In week 5, IDEXX and PrioCHECK detected seroconversion in 41.6%, VetExpert in 16.7% and BioChek and INgezim in 8.3% of samples. In week 9 PrioCHECK and IDEXX still detected seroconversion in, respectively 3 and 1 samples out of 12. The remaining tests recognized all samples as negative. In Farm II and III all ELISAs provided comparable results. Main differences were observed in 5 week old pigs. In Farm II IDEXX, INgezim and Vetexpert detected seroconversion in 83,3%, PrioCHECK in 75% and BioChek in only 16,7% samples. In Farm III, in week 5, IDEXX and PrioCHECK detected seroconversion in 93.8% samples and the remaining tests in 68.7-75% of samples. There were no differences between the kits in the number of seropositive pens in farms II and III. It shows that all tests are suitable for the general evaluation of herd status in PRRSV positive farms but observed differences in sensitivity may affect the detection of low level of seroconversion. The results of the specificity evaluation will be presented at the symposium.

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2

Complete genome sequencing of recently isolated Porcine Reproductive and Respiratory Syndrome Virus RFLP 1-7-4 strains reveals a familiar series of deletions in ORF1a that are similar to those seen in previously studied strains of high virulence

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RFLP pattern in their GP5 sequence. However, few studies have been done to examine the remaining portions of the genome of these strains. In this study, we sequenced the complete genome of four recently isolated strains of Porcine Reproductive and respiratory Syndrome Virus (PRRSV) is one of the most significant pathogens affecting swine production in the United States and the world over. The virus has evolved a significant amount of genetic and antigenic diversity that complicates the development of effective vaccines to combat disease. Recently, diagnostic laboratories and veterinarians have been reporting an increased incidence of virulent from within lineage 1, of which the MN-184 strain is also a member. A comparison of GP5 sequences to lineage 1 strains suggests that these isolates emerged from sub-lineage 1.5 with 1-7-4 strains exhibiting approximately strains of PRRSV that bear a 1-7-4 RFLP 1-7-4 PRRSV. We identified a series of deletions (relative to VR-2332) in the NSP2 portion of ORF1a in all four viral genomes that are reminiscent of those present in virulent PRRSV strains including MN-184 and NADC-30. We also compared the GP5 sequences of these isolates to a set of less **reference strains to determine which lineage of PRRSV they emerged from. All four RFLP 1-7-4 strains appear to have originated 96% identical to isolate 2002-1347 (sub-lineage 1.5), 91% identity to MN-184 (sub-lineage 1.9), and approximately 99% identity with one another. Divergence within ORF1a is even more pronounced with isolates, sharing identity with one** with

another and only sharing approximately 85% identity MN-184. In addition to our genetic and phylogenetic analyses, we also performed a challenge study to compare the virulence of one of the 1-7-4 isolates to two additional virulent PRRSV strains, NADC-20 and FL-12, in twelve week old pigs taken from animals two weeks post-challenge. Further analysis using microscopic histopathology and immunohistochemistry is currently underway. Our data concerning the genome sequence and phylogeny of these isolates may b. Respiratory lesions associated with the 1-7-4 strain were similar in severity to those observed from the FL-12 strain and were more severe than those observed in groups challenged with NADC-20 based on a gross examination of lungs e of use in continued surveillance for these strains in the field. Our challenge study data can be used to place **the virulence of a recent RFLP 1-7-4 isolate into context in comparison to other strains that have been studied previously**.

3

Simultaneous Detection of Swine Respiratory Pathogens Using a Multiplexed Detection Assay

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A critical challenge facing US agriculture in detecting and responding to outbreaks of swine respiratory disease is the availability of rapid and sensitive diagnostic assays for the detection of multiple diseases in a single assay. Laboratory methods currently used to detect animal diseases are generally single pathogen specific, time-consuming, labor-intensive, and difficult to scale up to meet diagnostic demands in the event of an outbreak.

Recently, Lawrence Livermore National Laboratory (LLNL), in collaboration with Kansas State University (KSU), has developed a multiplex molecular assay for rapid and sensitive diagnosis of respiratory diseases in pigs. The assay utilizes the x-Map technology developed by Luminex Corp. available currently in many diagnostic labs. The assay presented is well suited for high-throughput routine surveillance of multiple pathogens and is cheaper than multiple single-plex assays currently in use. The embedded foreign animal disease (FAD) pathogen beads can be readily removed for routine screening of domestic diseases, and re-introduced into the panel when there is an increased need for FAD surveillance. The newly developed assay can detect and differentiate 10 swine domestic and FAD pathogens, including four bacterial pathogens- *Mycoplasma hyponeumoniae*, *Actinobacillus pleuropneumoniae*, *Haemophilus parasuis*, and *Streptococcus suis*, and six viral pathogens- Porcine Reproductive and Respiratory Syndrome Virus (PRRSV), Swine Influenza Virus (SIV), Porcine circovirus type 2 (PCV2), Pseudorabies Virus (PRV), African Swine Fever Virus (ASFV) and Classical Swine Fever Virus (CSFV). All pathogens in the assay are represented by one to five unique DNA or RNA signatures for increased sensitivity.

The performance of each signature including sensitivity and specificity was assessed by testing against a panel of targets and near-neighbor agents. The sensitivity of the assay was determined using the target bacterial or viral nucleic acids, and purified plasmid constructs containing the specific target DNA fragments. The limit of detection (LOD) for PRRSV was 0.55 pfu/mL, PCV2 was 3.6 pfu/mL, and SIV was 0.55 pfu/mL. Additional validation testing using experimentally infected and fielded samples is underway at KSU.

In summary, we have developed a sensitive and specific multiplexed assay for the simultaneous detection of two FADs and eight domestic swine pathogens. This respiratory disease panel is suitable for high-throughput surveillance and diagnostic testing of important domestic swine diseases and FADs.

4

Why PRRSV-multivaccinated sows are negative in ELISA?

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In naïve pigs, PRRSV modified-live virus (MLV) vaccines induce a rapid and robust seroconversion as detected in ELISA. In absence of new contact with the virus, the antibodies fade out in a variable period ranging from months to more than one year. In most breeding herds, PRRSV vaccination regimes start with gilts and then include periodic re-vaccination in order to maintain the immunity of the sows (e.g. blanket vaccination with a MLV every 3-4 months). Interestingly, both European and American swine veterinarians often report that a variable percentage of sows may become or remain negative by ELISA in spite of having being vaccinated several times. At present, it is unclear if this fact is related to the lack of compliance of vaccine administration, to factors related to the immunological response of some sows or are attributable to a failure of the ELISAs for detecting those animals, among other causes. The present study deals with the investigation of this problem. For that purpose, three farms (F1, F2 and F3) with a history of a high proportion of seronegative vaccinated sows were selected. In all three farms, adaptation of gilts was done by deliberate exposure to wild-type PRRSV. Afterwards, recall immunization with a type 1 MLV was performed every third month (4 doses/year, blanket vaccination). In each farm, 60 females (gilts and sows distributed by parities) were sampled before one of the recall vaccinations with a MLV vaccine and were bled again 21 after the vaccination. Sera were analysed by three commercial ELISAs: PRRS X3 (E1) (Idexx Laboratories), CIVTEST suis PRRS E/S (E2) (Laboratorios Hipra) and Ingezim PRRS Universal (E3) (Ingenasa). All samples yielding a negative result were re-tested in order to confirm that they were truly negative in the ELISA. When one sow resulted negative by all the ELISAs, neutralizing antibodies and total antibodies were determined by the viral neutralization test (VNT) and immunofluorescence in MARC-145 cell-cultures. The seronegative sows were also bled for obtaining PBMC to be used in the IFN-gamma ELISPOT aimed to evaluate the cell-mediated immunity. Agreement between present in all farms, but their proportion was strongly influenced both by the ELISA used and by other factors of the farm itself. Before vaccination, F1 had a relatively low percentage results in the different ELISAs (Cohen's Kappa) was from fair to moderate: E1-E2=0.66 (IC95% 0.55-0.76); E1-E3=0.63 (IC95% 0.52-0.73); E2-E3=0.52 (IC95% 0.39-0.64). ELISA-negative sows were of negative sows (3.4-6.8%, depending on ELISA); in contrast, a high proportion of negative results was observed in F2 and F3 (45 and 29.4% for E1; 26.7% and 18.3% for E2; and 41.7 and 18.3% for E3, respectively). After vaccination, the proportion of negative results slightly increased in F1 (8.5-10.2%) and decreased in F2 and F3 with all ELISAs. Particularly, in F2 almost all negative gilts seroconverted, indicating that the initial acclimation exposure had failed. Nevertheless, after vaccination some animals remained negative by all the used ELISA in all three farms: 1.8% (n=1) for F1 and F2, and 3.9 (n=2) for F3, all of them from parity ≥3. These animals also showed negative results for the total and neutralizing antibodies tests and for the ELISPOT IFN-gamma. These results may suggest apparently some sows are anergic or develop anergy after repeated vaccination. The role of such sows in maintaining the infection should be investigated. Taken together, the results suggest that negative results in multiple vaccinated sows are a combination of false-negative results in ELISA and truly negative sows. These later deserve further investigation.

5

H-index as a measure of PRRSV contribution to porcine pathogens' research

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The bibliometric indicator H-index measures the number of published papers (N) that have been cited N or more times. This value combines both the quantity and the quality (number of citations) of the publications and is widely considered as a factual and accurate parameter to quantify an individual's scientific research output. Recently, it has been also used to determine the relative impact of infectious diseases in humans. In order to measure the importance of PRRSV research in the scientific publications, the present study aimed to calculate the H-index of PRRSV publications and to compare it with other swine pathogens, with special emphasis on viruses.

The H-index was calculated for a total of 137 swine pathogens –*viruses* (n=52), *bacteria* (n=39), and *other pathogens* (n=46), including helminthes, protozoa, external parasites and fungi. The bibliographic software

package Web of Science (WOS) v.5.16.1 was used for this purpose. Searches (March 2015) were done using the terms: "porcine", "pig" or "swine", each infectious agent complete name, acronym, common names, their synonymous and the associated disease/s. Selected papers were revised one by one to ensure database accuracy. Pathogens were classified by their zoonotic potential. Total number of citations per paper included in the H-index and the percentages of change in H-indices over time measured from the first paper published for each pathogen were also calculated. Finally, papers in PRRSV H-index were analysed for the origin of the first author affiliation.

Overall, H-index mean for non-zoonotic pathogens was higher than that of zoonotic ones (18.5 ± 21.6 and 10 ± 20.2, respectively). In regards to the groups of pathogens, H-index mean was 24.2 ± 23.2 for *viruses* (range 1-95), 28.8 ± 21.9 for *bacteria* (range 0-106) and 10.1 ± 10.8 (range 0-40) for *other pathogens*. In decreasing order, the ten highest H-indices and their total number of citations were: rank 1-*E.coli* (106;24023), 2-PRRSV (95;16398), 3-PCV2 (85;14068), 4-Swine influenza virus (SIV) (79; 16008), 5-Classical swine fever virus (72;8689), 6-*Salmonella typhimurium* (66;9805), 7-*Actinobacillus pleuropneumoniae* (64;7740), 8-*Streptococcus suis* (62;10739), 9-Aujeszky's disease virus (58;4498), and 10- Foot-and-mouth disease virus and African swine fever virus (56;6702 and 56;4802, respectively). The highest H-index in *other pathogens* was for *Toxoplasma gondii* (rank 22, H=40, citations=4562). Focusing on the viruses, the full list of the ten with the highest H-indices was completed by: Pig endogenous retrovirus (55;7578), Porcine rotavirus (PoRV) (49;5049) and Hepatitis E virus (49;6392). When changes in H-indices over time were calculated, PRRSV and PCV2 can be considered as "hot topics" since their increases were the highest (4.07% and 5.32% per year, respectively); in contrast, SIV with an annual increase of 1.66% -the lowest among the swine pathogens- can be considered as a "sleeping beauty" -its H-index for pigs has remained virtually unchanged during more than 50 years until pandemic outbreaks in 2009-. Of 95 PRRS publications, 62.1% were from North America (54.7% USA and 7.4% Canada), 31.6% from Europe (9.5% The Netherlands, 6.3% Belgium, 5.3% Denmark, 3.1% Spain, 2.1% France, 2.1% UK, 1% Germany and 1% Poland), and 6.3% from Asia (5.3% China and 1% Singapore); none were from South-America, Oceania or Africa. Despite being a non-zoonotic and a relatively new virus, PRRSV has an extremely high impact and research interest among swine pathogens as H-index demonstrates.

6

Monoclonal antibodies against the spike glycoprotein of porcine epidemic diarrhea virus

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Porcine epidemic diarrhea virus (PEDV), a member of the family *Coronaviridae*, is a large (~28 Kb) positive sense RNA virus that causes severe diarrhea, vomiting and dehydration in pigs. The PEDV genome contains seven open reading frames encoding two polyproteins (ORF1a and 1b), one accessory protein (ORF3), and four structural proteins (S, E, M and N). The spike (S) protein is the major surface glycoprotein involved in virus attachment and entry, thus, is also the main target of host immune responses. The goal of this study was to develop and characterize monoclonal antibodies (mAbs) directed against PEDV spike glycoprotein. For this, mice were immunized with sucrose purified/UV-inactivated PEDV strain CO13. After a series of four immunizations, splenic cells were fused with myeloma cells lines and hybridomas selected in the presence of hypoxanthine-aminopterin-thymidine (HAT) medium. Thirteen hybridoma clones producing mAbs against PEDV S were identified. All mAbs recognized full length PEDV S expressed by a heterologous vector in cell cultures *in vitro*. Additionally, these mAbs reacted against PEDV infected Vero cell cultures, indicating their ability to recognize native spike protein expressed in the context of virus infection. All mAbs are currently undergoing further characterization aiming at identifying immunogenic regions and/or epitopes within the spike glycoprotein. Characterization of immunodominant domains of the spike protein may provide important insights on PEDV infection biology and immunity.

7

Production and characterization of monoclonal antibodies against emerging swine pestivirus

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Atypical porcine pestivirus (APPV) has been reported to emerge in the US swine herd recently. To prevent its potential outbreak, specific diagnostic reagents and assays are urgently needed. In this study, we generated a panel of monoclonal antibodies (mAbs) against putative E2 glycoprotein of APPV. E2 antigen was expressed as the recombinant protein in E. coli expression system. Western blot result confirmed that the antigen was specifically recognized by field serum samples from APPV-infected pigs. Subsequently, BALB/c mice were immunized with the E2 antigen and splenocytes of hyperimmunized mice were extracted and fused with mouse NS-1 cells. Hybridoma cells were established under the HAT selecting medium. Specific hybridoma clones secreting E2-specific antibodies were initially screened by immunofluorescence assay and ELISA, and monoclones were obtained by subsequent single cell cloning. A total of five mAbs against APPV E2 were obtained. Cross-reactivity with other pestivirus E2 antigens, including BVDV E2 and CSFV E2, was not detected. These mAbs provide a powerful tool for development of rapid diagnostic assays for early detection of APPV infection.

9

Development of Fluorescence Microsphere Immunoassay (FMIA) for the Detection of Swine IgG Using Protein A, G, A/G, and L

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Fluorescence microsphere immunoassay (FMIA) is a molecular diagnostic technology which can be used for the detection of multiple targets simultaneously with high sensitivity and specificity. This technology has been applied to the diagnosis of infectious diseases in human and animals. IgG has been commonly used as a capture antibody in FMIA and ELISA. Previous studies in our laboratory showed that, FMIA is a diagnostic tool to detect swine IgG antibodies to several Porcine reproductive and respiratory syndrome virus (PRRSV) and Porcine circovirus type 2 (PCV2) recombinant proteins, including the major nucleoprotein, N. Protein A, G, A/G and L are recombinant proteins of microbial origin that bind to mammalian immunoglobulins. However, the uses of these proteins as a capture antibody in FMIA are very limited. One limitation of the current FMIA is that it relies on IgG as a capture antibody that may sometimes produce high backgrounds. The objective of these studies are the development of multiplex FMIA for the detection of PRRSV and PCV2 specific IgG antibodies in serum samples with low background using antibody binding proteins A, G, A/G, and L. For the production of recombinant antigens, PRRSV N and PCV2 capsid proteins were expressed in *E. coli.* Purity of each protein was verified by SDS-polyacrylamide gel electrophoresis (PAGE). The target antigens were assembled into a single multiplex and tested against sera from swine infected with PRRSV or PCV2. The results showed that protein A, G, A/G and L are responsive to IgG in swine sera. Protein G and L are weakly positive in binding to IgG compare to protein A. The experimental results demonstrated that PRRSV IgG positive antibodies were detected by MFI values ~20,000 using protein A in swine sera with very low or no background. The detection of PRRSV and PCV2 by the inclusion of proteins A, G, A/G, and L in FMIA would be an alternative strategy for the detection of infection in swine.

10

Realizing the potential of simple molecular tools for field diagnosis of foot-and-mouth disease

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Accurate, timely diagnosis is essential for control, monitoring and eradication of foot-and-mouth disease (FMD), an economically important vesicular disease of cloven-hooved animals. Currently samples are tested

at reference laboratories, a lengthy process which can delay critical decision making. This delay to decision making can be significant, for instance during the UK 2001 FMD outbreak, a 'slaughter on suspicion' policy was introduced to bypass lengthy laboratory confirmation, leading to the over-reporting and unnecessary culling of susceptible animals. Portable reverse transcriptase real-time PCR (rRT-PCR) provides a realistic option for rapid, sensitive, *in situ* detection. Here we evaluate a robust "field-ready" lyophilised rRT-PCR assay using the T-COR 8™ (Tetracore, Inc) platform for the detection of FMD virus (FMDV). A concordance study was performed between the diagnostic gold-standard laboratory-based rRT-PCR and portable rRT-PCR (T-Cor 8™) and RT-LAMP (Genie® II) platforms. Assays performed on the T-COR 8™, using dilutions of FMDV O/UAE/2/2003, consistently showed equivalent or greater sensitivity comparatively to the gold-standard laboratory rRT-PCR and RT-LAMP. The limit of detection was calculated for all platforms using RNA standards, with the T-COR 8™ detecting down to 10^0 genomic copies, compared to 10^1 for the laboratory rRT-PCR and RT-LAMP. Therefore, this study demonstrates an important transition for FMDV-specific molecular assays into formats suitable for field diagnostic use.

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Application of a broad-spectrum microbial detection array for the analysis of pig pathogens

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Many of the disease syndromes challenging the commercial swine industry involve the analysis of complex problems caused by polymicrobial, emerging/re-emerging and transboundary pathogens. Currently circulating transboundary diseases, such as African swine fever and classical swine fever are constant threats and raise concern about the possibility of both intentional as well an unintentional introduction. The best assurance of timely identification for known and unknown threats is to employ techniques that can track known disease threats, as well as rapidly identify the introduction of new pathogens before they become established.

In this study, we employed a novel and comprehensive microbial detection technology, the Lawrence Livermore Microbial Detection Array (LLMDA) that was designed to detect >8000 species of microbes. We evaluated the utility of LLMDA to analyze the microbial composition in serum, oral fluid and tonsil samples from pigs co-infected with porcine reproductive and respiratory syndrome virus (PRRSV) and porcine circovirus type 2 (PCV2). The assay was compared to standard PCR methods for the detection of PRRSV and PCV2.

Under conditions of experimental infection, the array identified PRRSV and PCV2, but at a lower sensitivity compared to standard polymerase chain reaction (PCR) detection methods. The pen-based oral fluid sample was the most informative, possessing signatures from several porcine-associated viruses and bacteria, which may contribute to the severity of PRRSV and PCV2 diseases.

Since PRRSV and PCV2 are immunosuppressive, a second goal was to evaluate the presence of other agents that may contribute to disease. In addition to PCV2 and PRRSV, the LLMDA also detected other viral co-infections including porcine parainfluenza, astrovirus, and porcine stool-associated virus from oral fluid samples. Common bacterial co-infections detected by LLMDA are Streptococcus suis, clostridium sp., Staphylococcus sp., and Enterococcus sp.

This study demonstrates the utility of Lawrence Livermore Microbial Detection Array in routine clinical diagnostics and surveillance. Even though microarrays are not as sensitive as standard PCR assays, they create the opportunity to query hundreds of thousands to several million sequence-specific DNA signatures, all in parallel. As the cost of microarrays decrease, the application for use in routine diagnostics and disease surveillance in veterinary livestock is expected to increase, especially in the analysis of syndromes that result from polymicrobial interactions.

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PEDV shedding patterns and antibody kinetics in commercial growing pigs

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INTRODUCTION

The aims of this prospective longitudinal field study were to (1) describe the pattern of PEDV shedding and comparative sensitivity of alternative sampling methods in growing pigs as revealed by RT-PCR testing of individual pig fecal swabs, pen fecal samples, and pen oral fluids (OF); (2) describe the kinetics of PEDV antibody by ELISA (IgA, IgG) testing of individual pig serum and pen OF samples; and (3) establish cutoffs and performance estimates for PEDV "whole virus" IgA and IgG ELISAs (WV IgA or IgG ELISA).

MATERIALS AND METHODS

Samples to achieve these aims were collected from two production sites (one PEDV positive; one PEDV negative).
Farm 1 was a 52-pen WTF barn stocked with ~800 pigs. Pen samples (feces and OFs) and pig samples (fecal swabs and serum) were collected from the same 6 pens and a convenience sample of 5 pigs in each of the 6 pens at placement and at ~2-week intervals for 27 weeks. At ~13 weeks of age, this PEDV-negative population was exposed to PEDV using standard field exposure techniques.
Farm 2 consisted of 3 identical 40-pen WTF barns, each stocked with ~900 pigs. Pen OF samples were collected from 36 pens (4 pens were not stocked) in each of the 3 barns and serum samples were collected from a convenience sample of 20 pigs in 2 pens (10 pigs per pen) in each barn. Sampling began at placement and was done weekly for a total of 9 samplings.
Pen feces, OF specimens and fecal swabs were tested by PEDV RT-PCR; OF and sera were tested by PEDV WV ELISA (IgG, IgA) using procedures available for routine testing at the ISU Veterinary Diagnostic Laboratory.

RESULTS

On Farm 1, PEDV was detected by RT-PCR at the first sampling post inoculation (DPI 6) in individual fecal swabs, pen fecal samples and pen OF. The last RT-PCR positives were detected in fecal swabs and OFs on 69 DPI. Overall, the highest percentage of positive samples was observed in OF. IgG and IgA responses were detected in the OF and serum samples collected at 13 DPI. The OF IgA response continued to increase through 97 DPI, while serum IgA responses peaked at 27 DPI.
Farm 2 remained RT-PCR negative throughout the monitoring period, i.e., these samples provide a source of negative samples for calculating cutoffs and performance estimates for the WV IgA and IgG ELISAs (analysis in progress).

CONCLUSIONS

Antibody (IgG and IgA) kinetics in oral fluids and comparison with the serum antibody responses has not previously been reported. Future work will focus on establishing antibody levels associated with cessation of PEDV shedding and/or protection against infection.

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PrioCHECK™ PRRSV VIA, an indirect ELISA for the discrimination of Type I from Type II PRRSV infections

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PRRS is probably the most important swine disease of the last half-century and thus control and eradication of PRRS virus continues to have a high priority for the global swine industry. Due to the complex nature of the disease, single diagnostic tests may not be enough to successfully manage PRRS virus infections but rather broadly applicable solution strategies are needed to effectively control the disease.
Here we present data for PRRSV disease management using the ELISA PrioCHECK PRRSV VIA. The PrioCHECK PRRSV VIA is an indirect ELISA being able to discriminate Type I (EU) from Type II (US) PRRSV infection by measuring the presence of neutralizing antibodies in serum of pigs. As different antigens for PRRSV Type I and Type II are used every serum sample has to be analyzed in duplicate.

In a field study with a total of 182 serum samples relating to 19 different pig herds the PrioCHECK PRRSV VIA correctly identified 17 herds relating to a diagnostic sensitivity of 89.4 % (herd level). The analytical specificity of Type I positive samples on Type II coated plates was calculated on individual serum samples derived from vaccinated animals. Analyzing individual serum samples of Type I positive pigs resulted in no false positive result hence relating to an analytical specificity of 100%. The same was found for Type II positive samples on Type I coated plates. The diagnostic specificity calculated on 312 samples derived from pigs with confirmed negative PRRSV status resulted in 99.3% for Type I coated plates and 99% for Type II (US) coated plates.

In a case study, pigs showed clinical signs for PRRSV despite regular vaccination with a Type I (EU) vaccines. PRRSV screening ELISA data revealed that all samples were positive. PrioCHECK PRRSV VIA results detected very high antibody titers to Type II (US) in more than 90% of the animals. In contrast low antibody titers to Type I (EU) were seen in 2 of the 17 animals. These results indicate that protection to heterologous PRRSV strains failed. This finding could be confirmed by positive Type I and Type II PRRSV PCR. As a consequence the veterinarian decided to change the vaccine in this farm.

In a second investigation serum samples of 10 boars known to be continuously infected with Type I (EU) PRRSV were tested with the PrioCHECK PRRSV VIA. All samples were negative to Type I (EU) PRRSV indicating that no neutralizing antibodies against Type I were present. In contrast 50% of the samples revealed high antibody titers to Type II (US) PRRSV. The same samples were also positive in the screening ELISA, PrioCHECK PRRSV Ab porcine. Type II (US) positive results could be confirmed by PCR indicating that the herd was affected by a fresh infection of Type II (US).

In summary we show that the PrioCHECK PRRSV VIA is a very reliable tool to discriminate Type I (EU) from Type II (US) PRRSV infections on herd level with an excellent diagnostic specificity. Furthermore the PrioCHECK PRRSV VIA can support a vaccination strategy and therefore help to minimize negative impact of a PRRSV infection as well as improve herd performance.

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A fast and cost-effective method for Whole Genome Sequencing (WGS) of PRRSV type 2 directly from field samples in China

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Objective

The objective of the current study is to find a fast and cost-effective method for whole genome sequencing (WGS) of Porcine Reproductive and Respiratory Syndrome Virus Type 2 in China.

Methodology

A total of 400 porcine lungs, serum, and swabs were collected from pigs that showed reproductive and/or respiratory failure in 10 provinces of China from November 2013 to May 2015, and 96 PRRSV type2 positive samples were chosen for whole genome sequencing. After all the RNA was extracted, full-length cDNA synthesis was performed with the SuperScript III First-Strand Synthesis System using a specific set of primer. PCR amplifications of PCR products covering the PRRSV genome in 2 fragments and 4 fragments were performed, and then PCR products were purified and quantified. After preparing the library, all PCR products covering 96 PRRSV whole genomes were sequenced under one single NGS run.

Result

The long range PCR products covering the whole genomes include 5' -UTR and poly(A)-tail of PRRSV type 2, among others; furthermore, most of the PCRs produced a single amplicon with the expected size. 64 samples were used for amplification of PCR products covering the PRRSV genome in 2 fragments, while 32 samples were used for amplification in 4 fragments. 57 PRRSV whole genomes were successfully assembled, ranging from 14716 to 15335 nucleotides (excluding the poly(A)-tail). Further analyses of the full genome sequences are in progress.

Conclusion

We present here a fast and cost-effective method for WGS of Porcine Reproductive and Respiratory Syndrome Virus (PRRSV) Type 2 directly from field samples in China, without the need for propagation in cell cultures, which is time consuming and can lead to further mutations.

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Tracking of African swine fever outbreaks by analysis of ASFV genomic variability within intergenic region I73R/I329L

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African swine fever is a highly contagious viral disease of pigs and wild boars. Devastating ASF outbreaks and the continuing epidemic in the Caucasus region and Russia (2007 – to date) underscore the significance of this disease and the threat it poses for pig production. Currently, molecular epidemiology of ASFV is based on phylogenetic analysis of the C-terminus of the major capsid protein, p72 (B46L gene), the p54 gene and the CVR within the B602L gene (Vir. Genes, 2009;38, 85-95). Using this ASFV genotyping protocol, all ASFV strains from the Caucasus region, the Russian Federation and EU were found to be identical, all belonging to ASFV genotype II (Vet. Micro, 2012;148, 415-419). and pages). However, additional genetic variation among ASFV isolates circulating in the Russian Federation and the European Union has been described by Gallardo et al, 2014 (EID, 2014; 20, 1544-7). Notably, ASFV intergenic region between I73R/I329L genes (TRS) appears to be a suitable genetic marker for ASFV molecular epidemiology that may

allow for differentiation of closely related ASF virus isolates.

The objective of this study was to evaluate the suitability of the ASFV TRS genetic marker as a complementary tool for molecular epidemiology of ASFV. To determine TRS genetic variation of ASFV isolates from different geographic regions, the intergenic region I329L/I73R was PCR amplified (Gallardo et al, 2014) and sequence aligned with ClustaW. Spatial and temporal patterns were evaluated using ArcMap with ArcGIS software 10.1(Esri CIS Limited, Moscow, Russia). ASFV isolates (N=70) from 18 regions across the Russian Federation (RF) originating from 2012-2015 were examined and mapped according to geographical location of outbreak origin. Results, based on insertion/deletion in TRS intergenic region, demonstrate the presence of two ASFV genetic variants. The relative number of ASFV isolates with additional insertion within TRS was higher from 2012 onwards. No significant difference in TRS variation was observed for ASFV isolates originating from domestic pigs and wild boar. In terms of geographical location, most ASFV variants with additional TRS insertions were identified in the Central and Southern part of the RF. Interestingly, that the same ASFV variant was identified in Ukraine/2012, Belarus/2013 and in all EU ASFV isolates. In the central part of the RF, an area of active animal production, transportation and commerce, both ASFV TRS variants are present.

Recently, we have used TRS analysis successfully to trace ASF outbreaks from sites of primary introduction to secondary outbreaks within regions of the RF where ASF disease introduction and transmission remain complex and unresolved. Thus, TRS analysis provides an additional and potentially powerful tool for ASF molecular epidemiology in an endemic disease setting.

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Discovery and pathogenesis of porcine parainfluenza-1 in pigs in the United States

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Porcine parainfluenza virus-1 (PPIV-1) was first identified in 2013 in slaughterhouse pigs in Hong Kong, China. The pathogenesis or endemic potential of PPIV-1 has not yet been assessed nor has the virus been formally identified outside of China. Here, metagenomic sequencing was used to assemble two complete genomes of PPIV-1 from nasal swabs collected from swine in Oklahoma and Nebraska. The genomes were 91.18-96.21% identical to the previously published Chinese strains. Phylogenetic analysis of a 1720bp segment of the HN gene from 13 U.S. samples suggests moderate genetic variability between strains with nucleotide identities from 89.5-100%. Comparison of the PPIV1 U.S. and Hong Kong samples yielded an 84.8-96.4% identity. Further molecular analysis by real-time RT-PCR (qRT-PCR) identified 17 positive samples out of 279 (6.1%) lung homogenate, oral fluid, or nasal swab samples from nine states from pigs with acute respiratory disease. Eleven nursery pigs from a naturally infected herd were monitored for virus replication and pathogenesis. No clinical signs of illness were apparent however qRT-PCR detected PPIV-1 in nasal swabs from seven pigs and the lungs of one animal. In situ hybridization identified PPIV1 RNA in the nasal respiratory epithelium and trachea to a lesser extent. Serological analyses using immunoprecipitation coupled to PCR detection and ELISA demonstrated seroconversion and further analysis of 60 swine serum samples resulted in 55.0% and 63.3% seropositivity, respectively. Taken together, the results confirm the widespread presence of PPIV-1 in the United States swine herd.

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Developing sampling guidelines for PEDV surveillance

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Oral fluids are a useful surveillance specimen because they are easily collected and can be tested for nucleic acids and/or antibodies to determine the infection status of the individuals contributing to the samples. Assays for testing swine oral fluid specimens have been available at veterinary diagnostic laboratories since 2010 and pen-based oral fluid sampling has become common practice for monitoring a variety of endemic pathogens of swine, e.g., PEDV, PRRSV, PCV2, IAV and others.

PEDV was identified in the U.S. in April 2013 by diagnosticians at the ISU-VDL. Shortly thereafter, a PEDV RT-PCR became available for routine testing and ~39,000 oral fluid samples and 47,000 fecal samples were tested between May 2013 - November 2014. Development of an IgG serum indirect ELISA for PEDV began in September 2013 and the assay became available for routine testing in September 2014. This ELISA is also available on request for the detection of IgA and/or IgG in oral fluids, colostrum, and milk.

The purpose of the study reported herein was to describe the spatiotemporal pattern of PEDV circulation in the field and to develop sampling guidelines. Oral fluid samples were collected from 36 pens (~25 pigs per pen) in 3 wean-to-finish barns on 3 sites for 8 weeks beginning one week post placement. ~2,916 individual oral fluid samples (108 pens per site x 9 sampling points x 3 sites) were tested for virus (RT-PCR) and antibody (IgG, IgA).

Analyses performed to date confirm the utility of oral fluid in surveillance and suggest a high degree of variability in the circulation of PEDV within and between sites, i.e., the distribution of positive pens and disease progression was unique to each individual barn. Mapping the viral movement on the study sites supports the conclusion that all barns must be sampled in order to establish disease status of a site.

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Developing sampling guidelines for oral fluid-based PRRSV surveillance

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Introduction

Oral fluids (OF) are a convenient surveillance sample because they are easily collected and can be tested for nucleic acids and/or antibodies for PRRSV and a variety of pathogens. We are currently developing statistically-based guidelines for sample size, frequency, and location.

Materials and Methods

Two studies were conducted to map the spatial and temporal aspects of PRRSV infection and further the development of OF sampling guidelines. **Study 1** - In one WTF barn on each of 10 production sites, OF samples were collected from 6 equidistant pens (~25 pigs per pen) every 2 weeks for 18 weeks. **Study 2** - In 3 wean-to-finish barns on one finishing site, OF samples were collected weekly from every occupied pen (108 pens; ~25 pigs per pen) for 8 weeks. OF samples were completely randomized and then tested for PRRSV RNA, IgG, and IgA. To date, statistical analyses have been done to examine spatial autocorrelation, compare detection based on systematic spatial vs random sampling, and compare sampling from the same pens vs alternate pens at each time point. Additional analyses are currently in progress.

Results

Analyses showed that the disease status of a pen in a barn was highly influenced by the disease status of other pens in the same barn, i.e., the presence of ≥ 1 positive pens increased the odds of detecting another positive pen (Table 1).

Table 1. Odds ratio (OR) for future pen PRRSV status based on current pen status	No. of positive pens in the barn (among 6 pens sampled)					
	0	1	2	3	4	5
OR that a positive pen will remain positive at the next sampling	NA	1.46	2.45	4.09	6.83	11.42
OR that a negative pen will test positive at the next sampling	0.27	0.45	0.76	1.27	2.12	3.54

Analysis has also shown that systematic spatial sampling was as good as or better than random sampling.

Discussion

The results of the analyses performed to date show that systematic spatial sampling is a viable approach for routine surveillance. Sample size calculations are in progress, but frequency of sampling is more important than sample size. That is, fewer samples collected at regular 2-week intervals are more useful that more samples collected at long intervals.

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Development of candidate Standard Operating Procedures for rapid and sensitive molecular surveillance of Foot and Mouth Disease (FMDv), Classical Swine Fever virus (CSFv) and African Swine Fever virus (ASFv) utilizing swine oral fluids

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Project summary: The primary objective of this project is to develop and validate a standard operating procedure (SOP) for the optimal purification and PCR detection of Foreign Animal Disease (FAD) from swine oral fluids (SOF). This study employed the comparative use of three commercially available magnetic bead extraction kits (GeneReach, USA; Qiagen, Germany; and Life Technology, USA) and various SOF samples. Phage surrogates kindly provided by Dr. McIntosh (FADDL) were used for Foot and Mouth Disease (FMDv), Classical Swine Fever virus (CSFv) and African Swine Fever virus (ASFv) and live virus stocks were used for Influenza A (pH1N1) and Porcine Reproductive and Respiratory Syndrome virus (PRRSv-JA142). Various concentrations of targets were spiked into SOF and extracted side by side using two automated extraction instruments (GeneReach Tacomini- 8 well and Qiagen BioSprint- 96 well). Protocols utilized were based on kit manufacture recommendations and/or protocol modifications reported by veterinary diagnosticians for use in routine surveillance using SOF. Extractions were performed in duplicate or triplicate, utilized varying volumes of SOF (100ul, 200ul, and 300ul) to evaluate the effect of input volume of SOF on the extraction efficiency or nucleic acid detection for the protocol optimization. Real time PCR detection of Influenza A, FMDv, and CSFv were performed per National Veterinary Service Laboratory protocol utilizing two commercially available RT-qPCR mastermixes (Life technologies-NVSL SOP reagents and Quanta Biosciences, USA-surge capacity reagents). Quanta Biosciences qPCR master mix (Fast mix II) was utilized for the detection of ASFv DNA. Detection of PRRSv RNA was performed using the Qiagen Virotype PRRSv kit. PCR was performed with the CFX 96 qPCR machine (Biorad, USA).

Results: GeneReach and Qiagen magnetic bead extraction kits yielded nearly equivalent nucleic acid (NA) detection for all five targets with greater than 90-99% recovery of NA utilizing 100-200ul of SOF in the extraction. PCR determined copy number for phage surrogates was generally highly reproducible as compared to live virus. In contrast, use of 300ul of SOF into the extraction resulted in markedly reduced recovery (0-45%) with high variability of PCR determined copy number for all targets especially when live virus was used. Results were independent of extraction instrumentation or PCR reagents. Poor recovery of

targets were observed with Life technology extraction kit. Nucleic acid recovery was reduced by approximately 2.5 logs resulting in only sporadic detection of targets at low concentrations (false negative). Additionally, magnetic beads from this kit were incompatible with the small scale extraction equipment, thus evaluation of this reagent was limited.
Conclusion: Preliminary studies demonstrate that optimal volume of SOF is critical for reproducible and sensitive detection of live virus. Additionally thorough evaluation of extraction methods is equally important. This works provides candidate SOPs and alternate PCR reagents for FAD surveillance using swine oral fluids.

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Characterization and application of monoclonal antibodies against porcine epidemic diarrhea virus

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Porcine epidemic diarrhea virus (PEDV) causes acute diarrhea to pigs at all ages, resulting in high mortality in piglets less than one week old. Since April 2013, PEDV has rapidly spread in the US and causes the loss of over 10% of the US pig population. Monoclonal antibody (mAb) is a key reagent for rapid diagnosis of PEDV infection. In this study, we produced a panel of mAbs against nonstructural protein 8 (nsp8), spike(S) protein, and nucleocapsid (N) protein of PEDV. Three mAbs were selected, which can be used in various diagnostic assays, including immunofluorescence assay, enzyme-linked immunoabsorbent assay (ELISA), Western Blot, immunoprecipitation (IP), immunohistochemistry (IHC) test and fluorescence in situ hybridization (FISH). The mAb 51-79 recognizes amino acid (aa) 33-60 of nsp8, and mAb 70-100 recognizes aa1371-1377 of S2 protein, while mAb 32-20 recognizes aa47-93 of N protein. Using the mAb70-100, the immunoprecipitated S2 fragment was examined by protein N-terminal sequencing, and cleavage site between S1 and S2 was identified. In addition, this panel of mAbs was further applied to determine the infection site of PEDV in the pig intestine. IHC test result showed that PEDV mainly located at the mid jejunum, distal jejunum and ileum. Results from this study demonstrated that this panel of mAbs provides a useful tool for PEDV diagnostics and pathogenesis studies.

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Porcine reproductive and respiratory syndrome virus non-structural protein Nsp2TF down-modulates Swine Leukocyte Antigen class I (SLA class I) expression

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Porcine reproductive and respiratory syndrome virus (PRRSV) is an economically important swine pathogen which typically induces sub-optimal immune responses thereby leading to a persistent infection. The sub-optimal immune responses against PRRSV are partly due to a delayed induction and impaired effector function of the cell-mediated immunity (CMI). Previous studies showed that PRRSV reduced the surface expression of swine leukocyte antigen class I (SLA-I) in susceptible host cells such as macrophages and dendritic cells, which may be partly responsible for the impaired CMI responses against PRRSV. In this study, we first verified the previous observations in a porcine kidney epithelial stable cell line expressing CD163 (PK15-CD163) infected with a PRRSV strain VR2385. Subsequently we investigated the PRRSV proteins responsible for SLA-I modulation and demonstrated that the viral proteins Nsp1a, Nsp2TF and GP3 significantly down-regulated the surface expression of SLA-I, with Nsp2TF showing the greatest potential of reduction. To further confirm whether Nsp2TF reduces SLA-I expression in the context of viral infection, a mutant virus (PRRSVΔNsp2TF) was generated using a DNA-launched PRRSV infectious clone in which the frameshifting elements that produced Nsp2TF were completely disrupted to knock out its expression. Additionally, to reinforce the knockout, several mutant viruses were generated with the introduction

of additional stop codons in the TF domain of the protein. The one with 3 additional stop codons (PRRSVΔNsp2TF-3UAGs) reversed SLA-I surface expression when compared to the wild-type virus infection. The results indicate that Nsp2TF down-regulates SLA-I expression during PRRSV infection. The results from this study identified a novel function for Nsp2TF in the negative modulation of SLA-I expression. Since antigen presentation associated with SLA-I molecules is essential for the activation of or recognition by T cells, the Nsp2TF may directly play a role in PRRSV-mediated immune modulation. Therefore, disrupting the Nsp2TF's ability to affect the SLA-I expression may improve the existing PRRSV vaccines towards a better CMI response against the virus.

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Differences in pathogenesis and immune responses between attenuated and virulent African swine fever virus strains

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African swine fever virus (ASFV) is the etiological agent of a contagious and often lethal viral disease of domestic pigs. There are no vaccines to control African swine fever (ASF). Experimental vaccines have been developed using genetically modified, live-attenuated ASFV obtained by specifically deleting viral genes involved in virulence. We used an experimentally attenuated strain, a 9GL-gene-deleted Pretoria 4 mutant (Pr4Δ9GLv) and its parental virulent strain, Pretoria (Pr4v), to analyze differences in pathogenesis and the induction of protective immune responses. By studying the pathogenesis of attenuated ASFV strains and the onset of the protective immunological response, we aim to better understand host mechanisms mediating protection. Swine (80-90lbs) inoculated intramuscularly with 10^4 hemadsorption doses (HAD50) of virulent Pr4v or attenuated Pr4Δ9GLv presented a clearly different pathogenesis. Pr4v-infected animals developed clinical signs as early as 3 days post infection, while Pr4Δ9GLv infected animals remained clinically normal during the entire 10-day observational period. Two animals from each group were euthanized and necropsied on 2, 4, 6, 8, and 10 days post infection (dpi). Virus detection in various lymphatic organs demonstrated the generalized presence of Pr4v, while Pr4Δ9GLv was only transiently detected in spleen and liver. The onset of protective immunity in Pr4Δ9GLv-inoculated swine was then assessed in detail. Groups of swine ($n \geq 10$) were infected with Pr4Δ9GLv and challenged 7, 10, 14, 21, or 28 days later with 10^4 HAD50 of Pr4v. Swine inoculated with Pr4v were either found dead or humanely euthanized between 6 and 15 days post infection. Thirty percent of the swine survived challenge after 7 dpi with Pr4D9GL, while 70 to 80% of the swine survived challenge at 10, 14, and 21 dpi. All animals challenged at 28 dpi with Pr4Δ9GLv were clinically protected against the challenge with parental virulent virus Pr4v. This indicates that some of the protective host mechanisms induced by Pr4Δ9GLv are already present by 7 dpi and progressively increase until day 28 post infection. ASFV-specific antibodies (detected by ELISA and IP) and peripheral blood mononuclear cells (PBMCs) that produce interferon-gamma after ASFV stimulation (detected by ELISPOT) were assessed pre- and post-challenge to identify host responses correlating with protection. The mere presence of ASFV-specific interferon-gamma-producing PBMCs and/or virus-specific antibodies on the day of challenge does not predict survival after challenge. We are currently extending these studies assessing the patterns of host gene activation in the protected animals.

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Both CD4+ and CD8+ T cells effectively suppress PRRSV replication in monocyte-derived macrophages

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Porcine reproductive and respiratory syndrome virus (PRRSV) is a difficult-to-control pathogen, due to extraordinarily high mutation and antigenic variation among global isolates, with two distinct genotypes and several subtypes in each genotype. Live attenuated vaccines induce partial protection including reduced plasma viremia and clinical disease before a delayed appearance of weak neutralizing antibody responses in pigs, suggesting a contribution of cell-mediated immunity (CMI). However, efficacy and mechanisms of CMI against diverse PRRSVs need to be defined using a physiologically relevant assay. Herein, a novel 7 day CMI assay was developed using a low dose of $PRRSV_{SD23983}$, monocyte-derived macrophage (MDM) targets, and T lymphocyte effectors. This assay was used to test the hypothesis that PRRSV-specific cytotoxic T-cells are the protection correlate in PRRSV-infected pigs. Sow 2 PBMCs stimulated for 7 days with $PRRSV_{SD23983}$ reproducibly suppressed PRRSV replication in 95.5-99.6% of PRRSV-susceptible autologous MDMs and MHC-matched heterologous MDMs. The suppression efficacy of sow 2 PBMCs was insignificantly reduced after depletion of CD8+ T cells; 95-98.3% of PRRSV-susceptible MDMs by CD8-depleted PBMC. Sow 1 and 3 PBMCs suppressed PRRSV replication in autologous MDMs and MHC-matched heterologous MDMs at 83.7-90.1%, but their suppression efficacies were partially reduced to 44.4-60.6% after CD4 or CD8 depletion. Surprisingly, a highly effective suppression by sow 2 effectors was also possible in MDMs from a MHC-mismatched sibling sow, suggesting low stringency MHC restriction in epitope presentations on MDMs with different MHC class-II haplotypes. The data in this study strongly supports that PRRSV-specific CD8+ and CD4+ cells effectively suppress PRRSV replication in PRRSV-infected sows with different haplotypes.

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Both $CD4^+$ and $CD8^+$ T-cells recognize porcine reproductive and respiratory syndrome virus epitopes and lyse infected macrophages in a biphasic mode

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Porcine reproductive and respiratory syndrome virus (PRRSV) is an economically important, difficult-to-control pathogen due to an extraordinary mutation rate, high antigenic variation, and immune modulation in infected pigs. Pigs immunized with live attenuated vaccines induce partial protection without significant neutralizing antibody responses, suggesting potential contribution of cell-mediated immunity (CMI). However, the efficacy and mechanism of CMI responses are still needed to be defined using a physiologically relevant and sensitive assay. We hypothesized that cytotoxic T-cells control PRRSV in pigs. As a tool to test this hypothesis, a cytotoxicity assay (CA) that measures the cytolytic activity of PRRSV-specific T-cells was developed using $PRRSV_{SD23983}$, monocyte-derived macrophage (MDM) targets, and T-cell effectors. Using the CA, the features of protective cytotoxic T-cells (CTL) were defined as follows. First, T-cells in re-stimulated sow PBMCs reproducibly delivered granzyme-B to PRRSV-infected MDMs. Second, T-cells contributing to cytolysis of PRRSV-infected MDMs had both $CD4^+$ and $CD8^+$. Third, both phenotypes of CTLs could recognize PRRSV epitopes presented from incoming virions before *de-novo* synthesis of PRRSV proteins after RNA transcription. Fourth, the major contributors to the biphasic cytotoxicity kinetics against PRRSV-infected MDMs were $CD4^+CD8^{+high}$ and $CD4^+CD8^-$ cells in early and late cycles of PRRSV replication, respectively. These results may be valuable for developing an efficacious T cell-focused vaccine which can effectively control infections with diverse PRRSV strains.

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T lymphocytes induced after infection with a single PRRSV strain recognize epitopes processed from highly diverse PRRSV strains

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Live attenuated virus vaccines for porcine reproductive and respiratory syndrome virus (PRRSV) induce partial protection with moderate reductions in viremia and clinical disease, even before the appearance of weakly neutralizing antibodies in pigs, suggesting the contribution of cell-mediated immunity (CMI). However, PRRSV-specific CMI responses need to be defined before being considered as a broadly protective mechanism against diverse PRRSVs. Herein, the breadth of CMI responses in pigs at different ages were analyzed against various PRRSV strains and proteins using T cells induced by each of two type II PRRSV strains. The hypothesis was that PRRSV-specific CMI responses induced by a PRRSV strain can recognize diverse isolates, overcoming limited homologous protection due to antigenic variations. An enzyme-linked immune-spot (ELISpot) assay using 12 PRRSV strains and overlapping-peptides encompassing all open reading frames of $PRRSV_{SD23983}$ was utilized to evaluate CMI responses in pigs (sow, 8-week-old pigs and 2-week-old piglets) infected with SD23983 or VR-2385 strain.

T cell responses from all sows (n=3) recognized antigenically diverse PRRSVs (>100 spot-forming unit), as well as multiple proteins of homologous PRRSV (SD23983 strain). Similarly, seven of ten 8-week-old pigs showed broad CMI responses against various PRRSV strains after challenged with VR-2385 strain. In contrast, only two of nine 14-day-old piglets intrauterine-challenged with SD23983 strain had strong and broad CMI responses. It is noteworthy that neutralizing antibody responses in the sows and piglets with broad CMI responses are only specific to the homologous PRRSV strain. These results suggest that the induction of CMI responses that recognize diverse isolates may contribute to overcoming the problem of suboptimal heterologous protection by conventional vaccines.

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Investigating the progression to adaptive immunity after infection with Porcine Reproductive and Respiratory Syndrome Virus

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Although the adaptive response to infection with Porcine Reproductive and Respiratory Syndrome virus (PRRSV) has been analyzed *in vivo*, there has not yet been an explanation for the delay in the occurrence of IFN-y secreting cells. Given that PRRSV is able to infect macrophages as well as CD163+ dendritic cells (DCs), it seems plausible that different antigen presenting cells (APCs) will evoke specific lymphocyte proliferative responses. We hypothesize that macrophages are responsible for the delay in immune protection, whereas DCs are responsible for stimulating IFN-y secreting lymphocyte proliferation. Using GMCSF-derived macrophages and Flt3L-derived bone-marrow dendritic cells (BMDCs), we first compared these cells in vitro in response to infection with PRRSV. We found that both MHCI and MHCII expression on the surface of macrophages were decreased in response to PRRSV after 24 hours of infection. On the other hand, we observed an up-regulation of MHC molecules on CD163+ DCs, suggesting their importance in the progression to adaptive immunity. In comparison, the CD163- DCs showed a down-regulation of both MHC molecules. Recently, our investigation into the innate response of BMDCs and macrophages has shown that BMDCs do not up-regulate CCR7 in response to infection, whereas macrophages showed increased CCR7 expression. Upon coming into contact with an antigen, antigen-presenting cells begin to process said antigen for presentation to T cells. CCR7 expression results in the migration of activated APCs to peripheral lymphoid organs (in response to CCL19/CCL21) where they present antigen to T lymphocytes, resulting in the progression to adaptive immunity. To further characterize the progression to adaptive immunity during PRRSV infection *in vivo*, animals were infected with PRRSV VR-2385 and T cells were isolated over the course of

the infection on different dates. By co-culturing BMDCs (163+ and 163-) and macrophages (M1 and M2) with T cells from the respective dates, we analyzed the differences in the T cell response to antigen-presentation by either macrophages or DCs. We hypothesize that only the BMDCs will be able to stimulate a significant IFN-γ cell mediated response. This could provide a theory for the delay in cell-mediated immunity observed *in vivo*.

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Genomic regions associated with PRRS vaccination and co-infection of commercial nursery pigs with PRRS and porcine circovirus type 2b (PCV2b) virus

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The objectives of this research were to estimate the effect of the guanylate binding protein 5 (*GBP5*) region on host response of nursery pigs co-infected with PRRS and PCV2b, to evaluate the effect of this region on response to vaccination against PRRS virus (PRRSV), and to identify other regions associated with host response to co-infection with and without prior vaccination for PRRS. Since *GBP5* is involved in innate response to PRRS, it is hypothesized that this region will have a larger effect on PRRS viremia of non-vaccinated (non-vx) than vaccinated (vx) pigs following co-infection. We also hypothesized that unique genomic regions would be identified for vx versus non-vx pigs post co-infection.

The data analyzed for this study were from two PRRS Host Genetics Consortium trials of 200 commercial nursery pigs each. Pigs were from the same genetic source and pre-selected based on genotype at the WUR single nucleotide polymorphism (SNP) in the *GBP5* region: half AA and half AB. Previous studies showed that the "B" allele was dominant, resulted in increased growth, and reduced PRRS viremia under PRRSV-only infection. Upon arrival at Kansas State University, pigs were randomly sorted into one of two rooms and all pigs in one room received a modified live PRRS vaccine 28 days prior to co-infection with PRRSV and PCV2b and followed for 42 days. All pigs were genotyped using the 80K BeadChip. PRRS viral load (VL) prior to co-infection (Pre PRRS), PRRS VL post co-infection (Post PRRS), and PCV2b VL were calculated as the area under the curve (AUC) of serum viremia from -28 to 0, 0 to 21, and 0 to 42 days post-infection (dpi), respectively. Genome-wide association studies were performed by fitting each SNP as a fixed effect in an animal model using ASReml4, with litter and pen(trial) as additional random effects and trial and the covariates weight and age at vaccination as fixed effects.

The WUR SNP had the greatest effect on PRRS non-vx ($P<0.0001$), was also significant for Pre PRRS ($P=0.03$), and suggestive for PRRS vx ($P=0.07$), but not significant for PCV2 vx ($P=0.26$) or PCV2 non-vx ($P=0.40$). The effects of WUR on Pre PRRS VL and PRRS non-vx VL groups were not significantly different ($P=0.67$), but the effects of WUR on primary response VL for these groups was significantly greater than post-co-infection PRRS VL for vx pigs ($P<0.05$). Several additional regions were identified for Pre PRRS, Post PRRS, and PCV2b VL, with the largest effect located near the major histocompatibility complex for PCV2 vx VL. The top SNP in this region showed a significant interaction with vx ($P<0.0001$) for PCV2b VL.

In conclusion, the effect of WUR genotype was only significant for PRRS VL upon primary exposure to PRRSV (by vaccination or co-infection), supporting the role of this region in innate immune function. Distinct genomic regions were identified for PRRS and PCV2b VL of vx versus non-vx pigs, indicating that different genes are involved in host response following vaccination for PRRS. This research was supported by USDA-NIFA grants 2012-38420-19286 and 2013-68004-20362.

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Mutations in a highly conserved motif of nsp1beta protein attenuate the innate immune suppression function of porcine reproductive and respiratory syndrome virus (PRRSV)

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PRRSV nonstructural protein 1beta (nsp1beta) is a multifunctional viral protein, which involves in suppressing host innate immune response and activating a unique -2/-1 programmed ribosomal frameshifting (PRF) signal for the expression of frameshifting products. In this study, site-directed mutagenesis analysis showed that R128A or R129A mutation introduced in a highly conserved motif ($_{123}$GKYLQRRLQ$_{131}$) reduced the ability of nsp1beta to suppress IFN-beta activation and also impaired nsp1beta's function as PRF transactivator. Three recombinant viruses, vR128A, vR129A and vRR129AA, carrying single or double mutations in the GKYLQRRLQ motif were created. In comparison to the wild type (WT) virus, vR128A and vR129A showed similar growth ability, while vRR129AA mutant had reduced growth ability in infected cells. Consistent with the attenuated growth phenotype *in vitro*, the pigs infected with nsp1beta mutants had lower level of viremia than that of WT virus-infected pigs. In cell culture systems, all of the three mutated viruses stimulated higher level of IFN-alpha expression and exhibited reduced ability in suppressing ISG15 production, in comparison to that of WT virus. In pigs infected with nsp1beta mutants, IFN-alpha production was increased in the lungs during early time points of post-infection, which was correlated with increased production of IFN-gamma in those mutated viruses-infected pigs. These data indicate that PRRSV nsp1beta plays an important role in the modulation of host immune response. Modifying the key residues on the conserved GKYLQRRLQ motif could attenuate the virus growth and improve PRRSV specific immune responses.

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The Innate Immunity of IEC following PEDV Infection

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Introduction: Porcine epidemic diarrhea virus (PEDV), is an economically important swine enteric coronavirus causing vomiting, watery diarrhea, dehydration, decreased body weight, and high mortality in piglets. PEDV is mainly transmitted by the fecal-oral route, primarily infects intestinal epithelial cells; the mucosal immunity is the first and critical important defense line against these enteric infections. The intestinal epithelia not only provide the mechanical intestinal epithelial barrier protection, but also play important role in inducing of the innate immune response and regulating the adaptive immune response in response to viral infection. Though several commercial PEDV vaccines available, the vaccines don't effectively induce strong mucosal immunity. The study demonstrated that wild type PEDV elicited more intestinal mucosal immunity especially IgA antibody response than the attenuated PEDV, but the exact mechanism is unclear. This study focused the induced innate immune response of the intestinal epithelia following PEDV infection, which will provide helpful guidance to design new PEDV vaccines and illuminate the PEDV pathogenesis.

Materials and Methods: the expression of TLRs, Cytokines, and chemokines by intestinal epithelia IPEC-J2 were analyzed by real time RT-PCR, ELISA taking advantage of the establish PEDV infection in vitro model.

Results and Discussion: intestinal epithelial cells IPEC-J2 is a non-tumorigenic intestinal epithelial cell line isolated from unsucking pig jejunum epithelium, is now widely recognized as the intestinal mucosa model in vitro study. Although there is no apparent CPE, real time RT-PCR and indirect immunofluorescence confirmed that PEDV CV777 is able to infect and replicate in IPEC-J2. IPEC-J2 up-regulated pathogen-

associated pattern receptors TLR3, TLR7, TLR9, TLR10, and RIG-1 3-20-fold expression after infection, TLRs up-regulation was positively correlated with viral replication. PEDV infection increased the expression of chemokine CXCL10 more than 200 times, which will recruit inflammatory cells to the intestinal mucosa. PEDV infection induced intestinal epithelial cells to secrete IL-12, thereby promoting TH1 and natural killer (NK) cells responses. In addition, compared to vaccine strain PEDV CV777, virulent PEDV Lnct2a elicited higher expression of intestinal epithelial cells to secret more A proliferation-inducing ligand (APRIL), a critical cytokine to regulate IgA response. In short, PEDV infection can induce innate immunity, thus affecting the following adaptive immune response.

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Porcine reproductive and respiratory syndrome virus nonstructural protein 4 cleaves VISA to impair antiviral responses mediated by RIG-I-like receptors

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Porcine reproductive and respiratory syndrome virus (PRRSV) is one of the most significant etiological agents in the swine industry worldwide. It has been reported that PRRSV infection can modulate host immune response, and innate immune evasion is thought to play a vital role in PRRSV pathogenesis. In this study, we demonstrated that highly pathogenic PRRSV (HP-PRRSV) infection antagonized type I IFN responses and drastically suppressed interferon-stimulated gene expression induced by poly(I:C) in HP-PRRSV-infected cells. Notably, we found that PRRSV specifically down-regulated virus-induced signaling adaptor (VISA), a unique adaptor molecule that is essential for retinoic acid induced gene-I (RIG-I) and melanoma differentiation associated gene 5 (MDA-5) signal transduction. When expressed in mammalian cells, PRRSV nsp4 was able to down-regulate VISA and suppress type I IFN induction. Moreover, we verified that nsp4 inhibited IRF3 activation induced by signaling molecules, including RIG-I, MDA5, VISA, and TBK1, but not IRF3. Importantly, VISA was cleaved and released from mitochondrial membrane mediated by nsp4, which interrupted the downstream signaling of VISA. However, 3A mutant in the 3C-like protease active sites abolished its activity. Taken together, these findings reveal a novel strategy evolved by PRRSV to counteract anti-viral innate immune signaling, which complements the known PRRSV-mediated immune-evasion mechanisms.

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Immunopathogenic characterization of three CSFV Cuban isolates containing the G761R mutation within the B/C domain of the E2-gene

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In this study, we investigated the infection in domestic pigs caused by three CSFV Cuban isolates (Holg-2009: HE584533, PR11/10-2: FR821757 and Stgo-2011: HE584534) that were previously molecularly characterized within the B/C domain into the E2 gene. These isolates exhibited the G761R mutation which has been associated with the positive selective pressure from 2001 to 2011 under regular vaccination in the current Cuban epizootic. This finding suggested that the selection of this substitution would be linked to a potential adaptive viral advantage and was hypothesized as an important factor in the CSFV virulence that might induce variation on the clinical manifestations of the disease in the field. Considering the above premises, we assessed the immunopathogenesis of this three CSFV Cuban isolates during the first weeks after infection. In addition, we included pigs that were infected with different doses of Margarita strain, previously characterized to generate severe clinical signs used as the infection control, and one non-infected-control

group. In this regard, we evaluated the impact on the induction of the humoral response and its relation to the course of infection and the RNA CSFV viral load. The clinical score data showed that the PR11/10-2 isolate generated subclinical CSFV infection. Some of these piglets developed slight fever peaks during the first 10 days post-infection. Therefore, this isolate showed the lowest clinical score value. The Stgo-2011 isolate exhibited a statistically higher clinical score when compared with the other two isolates and a mortality rate of 75% before the end of the study. Despite sharing the same G761R mutation, different clinical presentation were generated by the three CSFV Cuban isolates. Hematological, pathological and virological data will be discussed. Detailed molecular studies including the entire E2-gene in parallel with pathogenic valuation in domestic pigs will be required to assess the contribution of the positively selected sites to the
virulence of CSFV isolates.

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Serum metabolomic profiling study of classical swine fever virus-infected pigs

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Metabolomics is a powerful technology for assessing the global changes of small molecule metabolites in a biological system and has the potential for discovering novel biomarkers and better understanding of the pathogenesis of a disease. Classical swine fever (CSF) is a highly contagious swine infectious disease with typical feature of high fever, severe depression, hemorrhage, leucopenia and alternative constipation and diarrhea. However, it remains unclear if small molecule metabolites contribute to the disease progression of CSF. In the present study, serum metabolomics of CSFV Shimen strain-infected pigs was performed by using ultraperformance liquid chromatography/electrospray ionization time-of-flight mass spectrometry (UPLC/ESI-Q-TOF/MS) coupled with pattern recognition methods. Compared to healthy control animals, differential metabolites were identified in CSFV-infected pigs at day 3 and day 7 post challenge. These changed metabolites are associated with several key metabolic pathways, including tryptophan catabolism and kynurenine pathway, phenylanaline metabolism, fatty acid and lipid metabolism, citric acid cycle, urea cycle, branched-chain amino acid metabolism, and nucleotide metabolism. In addition, we also observed the changes of several metabolites that are exclusively associated with gut microbiome. Comparison of differential metabolites obtained at day 3 and day 7 showed that the levels of upregulated fatty acids and other metabolites reached peak at day 3, when the rectal temperature of infected pigs also reached peak and almost half of whole blood cells were depleted. Thus, the changed metabolites may be closely related to the disease progression. This study presents the first report on serum metabolomics profiles of CSF and our results provide a new perspective for understanding the pathophysiological progression during CSFV infection.

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Identification of Factors Associated with Tonsil Virus Levels in Pigs Experimentally Infected with Porcine Reproductive and Respiratory Syndrome Virus

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Porcine Reproductive and Respiratory Syndrome (PRRS) is a costly disease that has been prevalent in the United States swine herds for over 25 years. PRRS Virus (PRRSV) has been shown to persist in tissues, such as the tonsil, in excess of 150 days after initial exposure. These persistent pigs can trigger a secondary outbreak or infect naïve pigs. Successful identification and removal of persistent pigs should help with containment of PRRS and improve herd health. The objective of this study was to identify factors associated with levels of PRRSV in tonsils at 35 or 42 days post infection (dpi), reported on the log scale (TV). This study used ~500 pigs spanning three trials whereby pigs were inoculated with PRRSV isolate NVSL-97-7895. We hypothesized that 1) tonsil virus levels are influenced by host genetic factors; 2) there is a relationship between serum viremia and tonsil virus levels; 3) serum cytokine levels play a role in controlling tonsil virus levels; and 4) animals with lower tonsil virus levels will be less impacted in their growth. All statistical analyses were conducted in ASReml using an animal model with a genomic relationship matrix constructed using 60k genotypes. Experiment, sex and parity were fitted as fixed class effects, and weight and age at infection were fitted as covariates. Animal, litter, and pen nested within experiment were fitted as random effects. The mean TV was 4.55, with a phenotypic standard deviation of 1.31. The estimated heritability and litter components of TV were 0.4±7.4% and 7.0±5.2%, respectively. Wood's curves were fitted to serum viremia levels periodically collected from 0-35 or 42 dpi, in order to capture serum viremia dynamics and fitted as covariates into the above model. Time to Peak (TP; P=0.55) and Peak Viremia (PV; P=0.22) were not associated with TV. A one day increase in the Time to maximal serum clearance rate (Tmax) was associated with a 0.07±0.03 increase in TV (P=0.003), and a one unit increase in maximal clearance rate (Vmax) was associated with 3.34±0.75 lower TV. A one unit increase in area under the Wood's curve 0-35 dpi (VL35) was associated with 0.017±0.007 higher TV. The area under the curve of log serum cytokine levels 0-35 were computed for CXCL8, IL-1beta, IFN-alpha, and CCL2, and fitted as covariates for a subset of ~200 animals. Only CXCL8 had a significant association with TV (P=0.04). Unexpectedly, a one unit increase in CXCL8 response was associated with a 0.02±0.009 higher TV, which is opposite from previous predictions. Weights were fitted as covariates in the model but no significant association between weight gain and TV was found (P>0.05). Our results suggest that rate of serum viremia clearance may predict persistent virus levels in the tonsils. The estimated heritability of TV was very low thus limiting the potential of using genetic selection on tonsil virus levels for removal of PRRSV persistent pigs. **This project was funded by Genome Canada, USDA-NIFA grant 2013-68004-20362 and National Pork Board grants #12-061 and #14-223. We would also like to acknowledge contributions from members of the PRRS Host Genetics Consortium.**

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Suppression of porcine epidemic diarrhea virus replication by inhibition of the JNK/SAPK and p38 MAPK signaling pathways

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Since a number of viruses manipulate host cell signal transduction pathways including the p38 mitogen-activated

protein kinase and the Jun NH_2-terminal kinase/stress-activated protein kinase (JNK/SAPK), the present study was initiated to determine whether such signaling pathways play a role in porcine epidemic diarrhea virus (PEDV) replication. We found that PEDV infection activates the p38 MAPK and JNK1/2 pathways. Although p38 activation was more effectively mediated in PEDV-infected cells than JNK1/2 activation upon virus infection, the maximal induction of phosphorylated p38 and JNK1/2 was observed similarly at 12-24 h postinfection. Notably, UV-irradiated inactivated PEDV, which is capable of allowing viral attachment and internalization but incapable of pursuing viral gene expression, failed to trigger phosphorylation of both p38 MAPK and JNK1/2, suggesting that PEDV replication is responsible for their activation. The activation of both kinases was markedly diminished in the presence of specific inhibitors, SB202190 for p38 and SP600125 for JNK1/2. Direct inhibition of p38 MAPK or JNK1/2 activation by each chemical inhibitor significantly suppressed PEDV replication by affecting viral RNA synthesis, viral protein expression, and progeny release. In addition, the inhibitory effect of SB202190 in PEDV replication was shown to be more remarkable than that of SP600125. We previously demonstrated that PEDV induces caspase-independent apoptosis through the activation of mitochondrial apoptosis-inducing factor (AIF) to facilitate viral replication and pathogenesis. Next, it was investigated whether p38 and JNK1/2 activation is associated with the PEDV-induced mitochondrial AIF-mediated apoptosis pathway. Independent treatment with each inhibitor did not inhibit PEDV-induced apoptotic cell death. Furthermore, the mitochondrial-to-nuclear translocation of AIF, a specific hallmark of PEDV-triggered apoptosis, was verified in the presence of each inhibitor by confocal microscopy analysis. These results indicate that SAPKs are not involved in the apoptosis pathway during PEDV infection. Taken together, our data suggest that the p38 and JNK1/2 signaling pathways play an important role in post-entry steps of the PEDV life cycle and beneficially contribute to virus replication.

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Effect of circulating antibody on the course of PEDV infection in neonatal pigs

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Objective Many questions remain regarding the role of maternal anti-PEDV antibody in the protection of neonates against PEDV. A "passive transfer model" was used in this experiment to investigate the impact of circulating antibody on the course of PEDV infection and neonatal health.

Experimental design 6 PEDV IFA-negative sows were acquired from a commercial swine farm at ~110 days of gestation. After farrowing, piglets (n=62; 2-to-3 days of age) were intraperitoneally (IP) administered 1 of 6 levels of concentrated PEDV antibody to achieve circulating FFN antibody titers of (<1:8, 1:5.3, 1:6.1, 1:8, 1:17.1, and 1:32). 24 h later, piglets were inoculated with PEDV (10^3 $TCID_{50}$/ml) and observed for 14 days. Piglets remained on the dam throughout the study. Clinical observations, piglet fecal samples, body weight, and body temperature were collected on daily basis. Serum samples were collected from piglets at DPI -1, 0, and 14, or at the time of humane euthanasia. Fecal samples were tested by PEDV rRT-PCR. Piglet serum samples were tested for PEDV IgG and IgA (ELISA) and for PEDV FFN antibody. The effects of treatment on the outcomes measured were analyzed using repeated measures ANOVA.

Results

- PEDV circulating antibody had no effect on piglet growth or the level/duration of PEDV shedding in feces.
- The presence of antibody modified the body temperature response in infected piglets, i.e., treated piglets recovered from hypothermia by DPI 4, whereas negative control piglets recovered on DPI 7.
- Negative control pigs had the lowest survival rate (9.1%) compared to other groups (20-50%).
- The presence of circulating anti-PEDV antibody suppressed the humoral response of inoculated piglets, i.e., piglets that received passive antibody had significantly lower levels of FFN, PEDV IgA ELISA, and PEDV IgG ELISA antibody at 14 DPI versus controls.

Conclusions The results suggested that circulating antibody affects some parameters of PEDV infection in neonates (body temperature response, higher survivability, lower humoral response), but not others (piglet growth, PEDV fecal shedding). These results have implications for the management of PEDV in commercial herds.

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Involvement of programmed cell death-1 axis in immune suppression associated with concurrent PRRSV and PCV2 infection

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Suppression of the immune response is thought to be crucial in the development of porcine circovirus associated diseases (PCVAD). Many possible mechanisms of immune suppression have been studied; however, the role of the programmed cell death-1 (PD-1) axis in PRRS and PCV2 infection and its contribution to PCVAD has yet to be determined. The PD-1 axis is an important research target because increased expression interferes with effective T-cell activity and proliferation during the immune response. In our studies, monocyte derived dendritic cells (MoDCs) were infected with PCV2 and PRRSV in vitro and evaluated for expression levels of programmed death ligand-1 (PD-L1), IL-10, CD86, swine lymphocyte antigen-1 (SLA-1), and swine lymphocyte antigen-2 (SLA-2). Both PCV2 and PCV2/PRRSV co-infection of MoDCs significantly increased PD-L1 expression, while SLA-1, SLA-2, and CD86 expression levels were significantly decreased by coinfection by PCV2 and virulent stains of PRRSV, but not by PCV2 alone. MoDC IL-10 expression was significantly increased by PCV2 and co-infection with PCV2 and virulent strains of PRRSV. We also showed that the PD-L1/PD-1 axis induces anergy, apoptosis, and increased numbers of Tregs in pigs. The population of porcine lymphocytes with normal PD-1 expression contained significantly higher percentages of anergic and apoptotic cells, as well as increased $CD4^{+}CD25^{High}FoxP3^{+}$ Tregs, when compared to lymphocyte populations where PD-1 expression was reduced. These findings support previous reports of development of a regulatory phenotype of dendritic cells following PCV2/PRRSV co-infection and indicate that the PD-1 axis may be involved in the immune dysfunction seen when a second pathogen is added to PCV2 infection.

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Attachment and replication of genotype 1 porcine reproductive and respiratory virus on bone marrow-derived dendritic cells

BMDC but also in $CD163^{-}$ cells. It has to be mentioned that the proportion of $CD163^{+}$ BMDC decreased over time. The effect of the infection in iBMDC was generally more severe with higher cell mortality compared to the infection in mBMDC. Moreover, at similar m.o.i. isolate 3249 replicated always faster in both iBMDC and mBMDC while isolate 3267 showed a slow replication kinetic compared to the other two isolates. These preliminary results indicate that for type 1 isolates, BMDC probably harbor alternative receptors or mechanisms of entry of the virus. Besides this, differences in the replication behavior of different isolates are noticed.

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For many years, porcine sialoadhesin (PoSn, CD169) together with CD163 have been considered the main receptors for *Porcine reproductive and respiratory virus* (PRRSV). Thus, the attachment of PRRSV has been thought to occur by the interaction of the viral GP5-M heterodimer with PoSn while the interaction of the tetramer G2-GP3-GP4-E and CD163 resulted in internalization and viral uncoating. Recently, the role of

these receptors have been discussed and probably, other molecules. In the present study, the role of PoSn and CD163 in the attachment and susceptibility of bone marrow-derived dendritic cells (BMDC) to genotype 1 PRRSV was evaluated. For this purpose, three isolates designated as 3249, 3262 and 3267 (previously characterized in vitro and in vivo in several studies) were used to infect either immature (i) or mature (m) bone marrow-derived dendritic cells (BMDC). Initially, BMDC were incubated for 60 min at 4°C with each isolate at a multiplicity of infection (m.o.i.) of 1. Thereafter, cells were fixed and stained with an adequate anti-PRRSV monoclonal antibody (1CH5, Ingenasa) and anti-PoSn (3B11/11, AbD Serotec) or anti-CD163 (2A10/11, AbD Serotec) labeled with *Alexa Fluor® 610* or *Alexa Fluor® 488* as needed. Cells were then examined by means of confocal microscopy. Isolates 3249 and 3267 attached to both $PoSn^+$ and $PoSn^-$ BMDC as well as to $CD163^+$ and $CD163^-$ cells in both mBMDC and iBMDC while 3262 attachment was apparently restricted to mBMDC. In a second experiment, cells were infected at a m.o.i. of 0.1 and were incubated for 48 h being collected at 0h, 12h, 24h and 48h of incubation. As before, replication occurred not merely in $CD163^+$

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Apoptosis induced by the replication of different genotype 1 porcine reproductive and respiratory syndrome virus

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Porcine reproductive and respiratory syndrome virus (PRRSV) is assumed to induce apoptosis although the precise mechanisms causing it and the role of apoptosis in the pathogenesis of the infection are not fully clear. The purpose of this study was to evaluate the apoptosis induced by three genotype 1 isolates designated as 3249, 3262, 3267 previously characterized in vitro and in vivo. In experiment 1, porcine alveolar macrophages (PAM) were infected at a multiplicity of infection (m.o.i.) of 1 with each of the selected isolates and cultured for 48h. Replicas of the cultures were collected at 0h, 12h, 24h and 48h. Double immunofluorescence staining for PRRSV and cleaved caspase-3 (Asp175) and further examination under the microscope showed that apoptosis occurred in both infected and uninfected cells, and the intensity of apoptosis increased before large dead cells appeared. Most cells infected with isolates 3249 and 3267 died after 24h of infection because of fast viral replication, while for isolate 3262 viral replication had slower kinetics and the majority of cells survived for 48h. The results were compatible with an induction of apoptosis by both the intrinsic and the extrinsic pathways but the extent of apoptotic cells differed between isolates. In experiment 2, immature (i) and mature (m) bone marrow-derived dendritic cells (BMDC) were infected as above but with a m.o.i. of 0.1. Similarly to PAM, apoptosis was observed in both infected and uninfected cells. All three isolates replicated in both iBMDC and mBMDC. However, for isolates 3262 and 3267, replication was 1 log higher in iBMDC than in mBMDC after 48h of incubation (as measured by titration of the cell culture supernatants in PAM). In experiment 3, iBMDC were infected with the mentioned isolates and with isolate 2988 (m.o.i. 0.01), and were incubated for 24h. Then, cells were subjected to Annexin V/propidium iodide (PI) double staining for flow cytometry. Cells infected with isolate 3267 showed the highest proportion of both Annexin V^+/PI^- (27.7%) and Annexin V^+/PI^+ (30.7%) apoptotic cells, followed by cells infected with 3249 while 3262 and 2988 infected cells showed negligible staining for Annexin V/PI. In a subsequent analysis, isolates 3262 –inducing interleukin-10 (IL-10) and tumor necrosis factor (TNF)-alpha secretion in BMDC- and isolate 2988 –inducing only IL-10 release- were used again to infect iBMDC but in this case neutralizing antibodies for IL-10 were added in excess (>10 mg/ml) to the culture medium. Addition of IL-10 neutralizing antibodies leaded to the development of apoptosis as shown by the proportion of Annexin V^+/PI^- (7.5%) in 3262 infected cells while had no effect on 2988 infected cells. In summary, genotype 1 PRRSV induces apoptosis through both intrinsic and extrinsic pathways in PAM and BMDC although differences are noticeable between isolates, especially for BMDC.

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Antigenic variations in hypervariable antigenic region 1 on classical swine fever virus E2 protein impacts antibody neutralization

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Introduction: The Chinese hog cholera lapinized virus (HCLV) (also called C-strain) belongs to the subgroup 1.1 of classical swine fever virus (CSFV). The C-strain has been regarded as the safest and most reliable vaccine used in China since 1950s. However, several reports indicate that recent field isolates have branched away from the vaccine C-strain as subgroup 2.1 and become dominant in China. Although CSFV consists of only a single serotype, antigenic differences do exist among various genotypes which can be differentiated by the reaction patterns with specific monoclonal antibodies (mAb). Thus, we used a monoclonal antibody 4F4 against the N-terminal half of E2 protein of CSFV HZ08 strain to analyze the antigenic variations between recent field isolates and the C-strain. A recombinant virus RecCE2AR1$_{HZ}$ containing the variable region recognized by mAb-4F4 was rescued from the C-strain backbone and used to produce hyperimmune sera in rabbits for examination of the changes of neutralizing antibody titers to viruses of different subgroups.

Materials and Methods: Swine testicle (ST) cells were grown in Minimum Essential Medium supplemented with 10% fetal bovine serum (FBS). The following CSFV strains were used: the subgroup1.1 vaccine C-strain was propagated and titrated in ST cells. Two subgroup 2.1 strains were originally isolated from spleens of naturally infected pigs and replicated in ST cells. Immunofluorescence with mAb-4F4 to different strains was tested by a protocol used routinely in our laboratory. Truncated CSFV E2 proteins were expressed in a modified baculovirus expression system and purified by Ni-NTA affinity column. A recombinant virus RecCE2AR1$_{HZ}$ that contains the hypervariable region AR1 of the E2 gene from a field isolate HZ08 was rescued from the C-strain based infectious clone after genetic modification. The infectious clone was transcribed *in vitro* using the T7 Megascript system and the RNA transcript was transfected into ST cells by electroporation.

Results: Immunofluorescence shows that the mAb-4F4 reacted with subgroup 2.1 strains, but not with the C-strain. The major antigenic region that contributes to the binding of mAb-4F4 was identified by substitution on the baculovirus vector of three hypervariable antigenic regions (AR1, AR2 and AR3) of E2 protein between the genotypes 2.1 and the C-strain. The mAb-4F4 showed reactivity only with the C-strain E2-based recombinant protein containing the AR1 region from strain HZ08 but not with those of AR2 or AR3 replacement. Furthermore, double mutations of the C-strain E2 protein with D705N and L709P in the AR1 region exhibited binding to mAb-4F4, indicating that N705 and P709 in the field strains are responsible for the reactivity. The mAb-4F4 showed good neutralizing activity to subgroup 2.1 isolates with its neutralizing value as high as $10^{3.81}$. The recombinant virus RecCE2AR1$_{HZ}$ showed similar growth to the C-strain, induced thermal response and specific neutralizing antibodies in rabbits. The neutralizing activity of hyperimmune rabbit sera raised with RecC-HZ-E2-AR1 to field isolates was significantly higher than the serum from C-strain vaccinated rabbits ($10^{3.77}$ *vs* $10^{3.38}$). Early studies have identified two independent antigenic units B/C and A/D (residues 690-800 and 766-865, respectively) that are located in the N-terminal half of E2 protein. The AR1 region recognized by the mAb-4F4 is also located in this region where there are other reported epitopes than the region containing aa705 and aa709 found in this study.

Conclusions: The mAb-4F4 specifically recognizes the AR1 region of subgroup 2.1 strains that contributes to neutralization. The two residues aa705 and aa709 are essential in forming the conformational epitope in this region. Replacement in the C-strain of the AR1 region of field isolates increased the neutralization titers to field viruses. The mAb-4F4 has the potential for differentiation between the vaccine C-strain and field isolates.

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Comparative analysis of signature genes in PRRSV-infected porcine monocyte-derived dendritic cells at differential activation statuses

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Activation statuses of monocytic cells including monocytes, macrophages and dendritic cells (DCs) are critically important for antiviral immunity. In particular, some devastating viruses, including porcine reproductive and respiratory syndrome virus (PRRSV), are capable of directly infecting these cells to subvert host immunity. We have recently profiled signature genes and gene responsive pathways in macrophages at different activation statuses, and reported that macrophage polarization is crucial for antiviral regulation. Monocyte-derived DCs (mDCs) are major target cells in PRRSV pathogenesis; however, the plasticity of mDCs in response to activation stimuli and PRRSV infection remains unstudied. In this study, we polarized mDCs using the framework established in macrophages, and applied genome-wide transcriptomic analysis to compare signature genes involved in mDCs activation and response to PRRSV infection. Our long-term goal is to integrate activation status with antiviral responses in these cells and to functionally modulate them for a prototypic cellular adjuvant/vaccine that is ideal for potentiating antiviral immunity. Porcine mDCs were polarized with mediators for 30 hours, then mock-infected, infected with PRRSV strain VR2332, or highly pathogenic strain (HP-PRRSV), for 5 h. Total RNA was extracted from the pooled cells of four replicates, and used to construct sequencing libraries for RNA-Seq procedures previously optimized. Comparisons were made between each polarized and unpolarized groups (i.e. mediator vs. PBS), and between PRRSV-infected and uninfected cells stimulated with the same mediator. The overall similarity between samples was assessed in heat map plots calculating the Euclidean distance between regularized log transformed data to allow equal contribution from all genes. Principal component analysis, Poisson distance and DESeq2 dispersion estimates emphasized variations in comparisons. Clustering of samples was by virus strain and then by mediator. We then asked which genes showed the most variability across all treatments as these are likely to be the genes that will provide resolution for clustering the samples. Many of the genes showing the most variability were related to cellular structure and innate immune response. The magnitude of differentially expressed gene profiles detected in HP-PRRSV rJXwn06 infected mDCs as compared to VR-2332 infected mDCs was consistent with the increased pathogenicity of the HP-PRRSV in vivo.

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Association of clinical outcome with microbiome and immunological response following co-infection with porcine reproductive and respiratory syndrome virus (PRRSV) and porcine circovirus type 2 (PCV2)

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Co-infections involving porcine reproductive and respiratory syndrome virus (PRRSV) and porcine circovirus type 2 (PCV2) are common and contribute to a range of polymicrobial disease syndromes in swine. Both viruses decrease the host immune response, increasing infections by primary and secondary pathogens. Clinical outcome following co-infection with PCV2 and PRRSV can vary from acute death to a complete lack of overt disease. To investigate differences in microbiome and immunological response associated with clinical outcome, 95 six-week old pigs were co-infected with PCV2 and PRRSV. At 70 days post-infection (dpi), 20 representative pigs were selected as having the best or worst clinical outcome based on average daily gain (ADG) and the presence of clinical disease. ADG for the worst clinical outcome group (0.475 ± 0.15 kg) and the best clinical outcome group (0.837 ± 0.04 kg) were significantly different ($p < 0.0001$). Worst clinical outcome pigs had prolonged and greater levels of viremia as

measured by qPCR. Mean PRRSV and PCV2 viremias were significantly higher on 28 dpi ($p < 0.02$) and 14 dpi ($p < 0.05$), respectively. Serum, lung and fecal samples collected at 70 dpi were analyzed using DNA microarray technology, which can detect over 8,000 microbes. Overall, microbe diversity was greater in the serum and lower in the feces of worst clinical outcome pigs. Detection of Proteobacteria in the feces, specifically *Escherichia coli* ($p = 0.03$), was associated with improved clinical outcome. This study provides insight into the effects of microbiome on clinical outcome following PRRSV and PCV2 co-infection, as well as highlights the importance of host response to virus challenge.

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Bioinformatic analyses of early host response to Porcine Reproductive and Respiratory Syndrome virus (PRRSv) reveals pathway differences between pigs with alternate genotypes for a major host response QTL

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A region on Sus scrofa chromosome 4 surrounding single nucleotide polymorphism (SNP) marker WUR10000125 (WUR) has been shown to be strongly associated with both weight gain and serum viremia in pigs after infection with PRRS virus (PRRSv). A proposed causal mutation in the guanylate binding protein 5 gene (*GBP5*) is predicted to truncate the encoded protein. To investigate transcriptional differences between WUR genotypes in early host response to PRRSv infection, an RNA-seq experiment was performed on globin depleted whole blood RNA collected on 0, 4, 7, 10 and 14 days post-infection (dpi) from eight littermate pairs with one AB (favorable) and one AA (unfavorable) WUR genotype animal per litter.

Gene Ontology (GO) enrichment analysis of transcripts that were differentially expressed (DE) between dpi across both genotypes revealed an inflammatory response for all dpi when compared to day 0. However, at the early time points of 4 and 7 dpi, several GO terms had higher enrichment scores compared to later dpi, including inflammatory response ($p<10^{-7}$), specifically regulation of NFkappaB ($p<0.01$), cytokine, and chemokine activity ($p<0.01$). At 10 and 14 dpi, GO term enrichment indicated a switch to DNA damage response, cell cycle checkpoints, and DNA replication. Few transcripts were DE between WUR genotype on individual dpi or averaged over all dpi, and little enrichment of any GO term was seen in these transcript lists. However, there were differences in expression patterns over time between AA and AB animals, which was confirmed by genotype-specific expression patterns of several modules that were identified in weighted gene co-expression network analyses (WGCNA). Small differences between AA and AB animals were observed in immune response and DNA damage response, but a more significant effect between genotypes pointed to a difference in ion transport/homeostasis and the participation of G-coupled protein receptors, which was reinforced by results from regulatory and phenotypic impact factor analyses between genotypes. We propose these pathway differences between WUR genotypes are the result of the inability of the truncated GBP5 of the AA genotyped pig to restrain viral entry and replication as fast as the intact GBP5 protein of the AB genotyped pig. Acknowledgements: USDA NIFA PRRS CAP Award 2008-55620-19132, USDA NIFA award 2012-38420-19286, the National Pork Board, the NRSP-8 Swine Genome and Bioinformatics Coordination projects, Genome Canada, Genome Alberta, the Alberta Livestock and Meat Agency and PigGen Canada, and the pig breeding companies of the PRRS Host Genetics Consortium (PHGC).

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Genomic prediction of the number of mummified piglets during a PRRS outbreak using PRRS antibody response during acclimation in health-challenged farms

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Previous analyses of data from a PRRS outbreak sow herd found that PRRSV antibody (Ab) response (PRRSV ELISA Sample-to-Positive [S/P] ratio) had a high negative genetic correlation with number of mummified piglets (NMUM) during the outbreak (-0.66 ± 0.28). In addition, genomic analysis showed that S/P is in part controlled by two Quantitative Trait Loci (QTL) on *Sus scrofa* chromosome (SSC) 7, one encompassing the Major Histocompatibility Complex (MHC) region and the other at ~130 Mb. The objective of this work was to evaluate the use of high-density Single Nucleotide Polymorphism (SNP) genotypes to predict S/P and NMUM following a PRRS outbreak, using SNP estimates based on S/P. The training dataset included data on 1,233 (100% PRRS-seroconverted) F1 replacement gilts that were sourced from 17 high-health multipliers from 6 genetic sources and introduced into 22 commercial farms with historical cases of natural disease challenges. Blood samples were collected 40.1±14 days after entry and used for semi-quantification of PRRSV ELISA Ab (S/P), while animals followed standard acclimation procedures. The validation dataset included 402 sows from the PRRS outbreak herd. Pigs were genotyped for 40K SNPs. The estimated effects for all SNPs on S/P were split into five scenarios: all SNPs across the genome (ALL_{SNP}), only SNPs in the two QTL ($SSC7_{SNP}$), only SNPs in the MHC QTL (MHC_{SNP}), only SNPs in the 130 Mb QTL (130_{SNP}), and all SNPs outside the two QTL ($Rest_{SNP}$). Accuracy of genomic prediction was calculated as the correlation of predicted S/P with S/P or NMUM in the outbreak herd, divided by square root of heritability. For prediction of S/P, all scenarios showed moderate accuracies, with the exception of $Rest_{SNP}$ (0.11), with the highest accuracy observed for $SSC7_{SNP}$ (0.38), followed by ALL_{SNP} (0.33), MHC_{SNP} (0.31), and 130_{SNP} (0.24). For prediction of NMUM, accuracies were greatest for ALL_{SNP} (-0.21) and $Rest_{SNP}$ (-0.36). Correlations of S/P predictions based on SNPs in the QTLs with NMUM were positive: 0.11 (MHC_{SNP}), 0.03 ($130b_{SNP}$), and 0.11 ($SSC7_{SNP}$). These results demonstrate that S/P in an outbreak herd can be predicted with moderate accuracy using SNPs located in the SSC7 QTLs. In contrast, greater genomic prediction of NMUM using S/P is observed using the rest of the genome and predictions of S/P based on SNPs in the SSC7 QTLs had an opposite relationship with NMUM than predictions of S/P based on the rest of the genome. Financial support from Genome Canada, the Canadian Swine Health Board, and PigGen Canada is appreciated.

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Evaluation of humoral immune status in porcine epidemic diarrhea virus (PEDV) infected sows under field conditions

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Porcine epidemic diarrhea virus (PEDV) is an economically devastating enteric disease in the swine industry. The virus infects neonatal suckling pigs, causes severe dehydration, and mortality rate is up to 100%. Currently, available vaccines are not completely effective and feedback methods utilizing PEDV infected material has variable

success in preventing reinfection. Therefore, comprehensive information on the levels and duration of effector/memory IgA and IgG antibody secreting B cell response in the intestines and lymphoid organs of PEDV-infected sows, and their association with specific antibody levels in clinical samples such as plasma, oral fluid, and feces is important. Our goal was to quantify PEDV specific IgA and IgG B cell responses in sows at approximately 1 and 6 months post-infection in commercial swine herds, including parity one and higher sows. Our data indicated that PEDV specific IgA and IgG levels in the plasma and oral fluid (but not feces) samples could be used for disease diagnosis purpose. PEDV specific B cell response in the intestines and spleen of infected sows decline by 6 months, and associates with the antibody levels in the plasma and oral fluid samples, but the virus neutralization titers in plasma remains high beyond six months post-infection. In conclusion, in sows infected with PEDV the presence of effector/memory B cell response and strong virus neutralization titers in plasma up to 6 months post-infection, suggests their potential to protect sows from reinfection and provide maternal immunity to neonates, but challenge studies are required to confirm such responses.

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Nsp1 and a part of Nsp2 genes of a synthetic porcine reproductive and respiratory syndrome virus are responsible for the viral capacity to induce type I interferons

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Type I interferons (IFNs) play a critical role both in innate resistance to viral infection and in regulating the host adaptive immune response. Porcine reproductive and respiratory syndrome virus (PRRSV) is well characterized for its ability to suppress the production of type I IFNs. We recently generated a synthetic PRRSV strain whose genome was rationally designed based on a set of 59 full genome sequences of type II PRRSV. This synthetic PRRSV-CON virus confers outstanding levels of heterologous protection as compared with the prototype PRRSV strain FL12. Unlike most of naturally occurring PRRSV strains, the PRRSV-CON virus induces robust levels of type I IFN response *in vitro*, rather than suppressing such response. The ultimate objective of this study is to identify which genes (or parts thereof) of this synthetic PRRSV-CON virus are responsible for inducing type-I IFNs. Through the use of both gain and loss of function studies, we found that the 5' end fragment of PRRSV-CON genome encoding non-structural protein (nsp) 1-alpha, 1-beta and a part of nsp2 genome is solely responsible for inducing type-I IFNs. We are in the process of further mapping individual viral genes involved in induction of type-I IFNs. Collectively, the results obtained from this study may be beneficial for the rational development of more effective PRRS vaccine.

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Genome wide association analyses of piglet response to experimental infection with one of two isolates of the Porcine Reproductive and Respiratory Syndrome virus

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Porcine Reproductive and Respiratory Syndrome (PRRS) is a devastating disease in the swine industry. Identification of host genetic factors that enable selection of pigs with improved performance during infection with PRRS virus (PRRSV) would reduce the economic impact of this disease. The objectives of this study were the identification and functional annotation of genomic regions associated with PRRSV response phenotypes. The study involves the genomic analysis of 13 trials, each with ~200 nursery age piglets that were experimentally infected with one of two isolates of the PRRSV (NVSL and KS06) and followed for 42 days post infection (dpi). Phenotypes analyzed were: viral load (VL) in blood during the first 21 dpi and weight gain (WG) from 0 to 42 dpi. Animals

were genotyped using the Illumina Porcine SNP60 Beadchip. Bayesian variable selection (Bayes-B) and single SNP association methods identified multiple genomic regions on Sus scrofa chromosomes (SSC) that were associated with VL and WG. Several regions were consistently identified by both Bayes-B and single SNP analyses, although many regions identified by single SNP analyses were not identified using Bayes-B. Bayes-B and single SNP analysis identified the same significant ($P<1x10^{-6}$) genomic regions on SSC 3 and 5 to be associated with VL in the KS06 trials and on SSC 6 in the NVSL trials; for WG, regions on SSC 5 and 17 were identified in the NVSL trials ($P<3x10^{-5}$). No regions associated with WG in the KS06 trials were identified with both methods. Additional regions were identified using either Bayes-B or single SNP analysis. Except for the *GBP5* region on SSC4, which was associated with VL for both isolates (but only with WG for NVSL), identified regions did not overlap between the two PRRSV isolate datasets, which disagrees with the high genetic correlations of traits between isolates estimated from this data by Hess et al. (2014). We then assessed the enrichment of GO annotation terms for genes located near SNPs that were associated with VL or WG at a relaxed significance threshold ($P<0.003$). We found that such genes were enriched for multiple immunologically related GO terms for VL and for metabolism related GO terms for WG. More functionally relevant candidate genes were identified by these analyses than by annotation of genes in close proximity to only the most significant effects. Thus, although use of single SNP analyses and a low threshold may increase the number of false positive results, it also increased the identification of true positives, as evidenced by the biologically relevant results we found through functional analysis of lists of genes near associated SNPs. Hess et al. (2014) estimated high genetic correlations of traits between isolates, but GWAS results did not reveal genomic regions consistently associated across PRRSV isolates. Furthermore, except for the SSC4 region, the genetic control of host response to PRRSV infection appears to be quite polygenic; many genomic regions have small effects on the traits. This project was funded by Genome Canada, USDA-NIFA grant 2013-68004-20362. We would also like to acknowledge contributions from members of the PRRS Host Genetics Consortium.

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CD16 is involved in the ADE of PRRSV infection

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The immunological effect of porcine reproductive and respiratory syndrome disease virus (PRRSV) vaccines is thought to be influenced by a variety of host factors, in which antibody-dependent enhancement (ADE) of infection is one crucial factor. In this study, we assessed the mechanism of ADE of PRRSV infection. First, we found that subneutralizing serum could induce ADE of PRRSV infection in porcine alveolar macrophages (PAMs). Quantitative PCR, Western blotting and flow cytometry revealed that CD16 (Fc gamma receptor III) is the most abundant Fc-gamma receptor expressed on the surface of PAMs; thus, the role of CD16 in ADE of PRRSV infection was examined in PAMs. By using functional blocking antibodies against CD16, we demonstrated that CD16 is involved in enhanced virus production in PRRSV–antibody immune complex-infected PAMs. Because PAMs co-express different Fc-gamma receptor isoforms, we evaluated the effects of CD16 in Fc-gamma receptor non-bearing cells (COS-7 and 293T) by transfection. Using these engineered cells, we found that CD16 could specifically bind to the PRRSV–antibody immune complex and subsequently mediate the internalization of the virus, resulting in the generation of progeny viruses. We also showed that efficient expression of CD16 required association of the FcR gamma-chain. Together, our findings provide significant new insights into PRRSV infection, which can be enhanced by CD16-mediated PRRSV–antibody immune complexes. This CD16-mediated ADE may induce a shift in PRRSV tropism towards CD16-expressing cells, distributing virus to more organs during virus infection.

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PEDV does not replicate in porcine monocyte-derived dendritic cells, but up-regulates the transcription of type I interferon and chemokine

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Porcine epidemic diarrhea virus (PEDV) infection causes significant economic losses to the swine industry worldwide. PEDV belongs to the type I coronavirus and is the major etiological agent of the recent outbreak of piglet diarrhea and death. So far, limited knowledge is available regarding the role of dendritic cells in PEDV infection. Here, we observed that PEDV doesn't replicate in monocyte-derived dendritic cells as evidenced by the decrease of viral gene transcript copies in infected cells and the absence of virus in the supernatants of infected cells over time. In addition, PEDV does not compromise cell viability at 48, 72, and 96 hours after infection at either a MOI of 2.5 or 5. Interestingly, an increased transcription of type I interferon including interferon-alpha and beta are observed in infected cells compared to mock infected cells. We also observe a dramatically increased transcription of chemokine IP-10. Surprisingly, we did not detect any interferon beta in the supernatants of infected cells. A slight increase in interferon alpha protein production in the supernatants of PEDV-infected cells was observed compared to mock infected cells. The surface expression of MHC I and MHC II is up-regulated in PEDV-infected cells compared to mock cells. We are currently assessing whether PEDV infection compromises the phagocytic activity of Mo-DC and whether PEDV-infected dendritic cells are capable of activating the proliferation of autologous T cells.

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PRRSV Infection Promotes STAT3 Degradation via Nsp5

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The typical features of immune responses in PRRSV-infected pigs are delayed onset and low level of neutralizing antibodies and weak cell-mediated immunity. It is known that lymphocyte development and differentiation rely on cytokines, many of which signal via JAK/STAT pathway to exert their biological effect. STAT3 (signal transducer and activator of transcription 3) is a signaling mediator of many cytokines including IL-6 and IL-10, and thus plays a key role in many cellular processes such as cell growth and in regulating host immune and inflammatory responses. The objective of this study was to evaluate the effect of PRRSV infection on STAT3 signaling. PRRSV infection of MARC-145 cells led to significant reduction of STAT3 in comparison to uninfected cell control. Several PRRSV strains tested showed similar suppressive effect on STAT3, however, real-time RT-PCR result showed that the transcript level of STAT3 had minimum change. The PRRSV-mediated STAT3 reduction was in a dose-dependent manner: STAT3 level deceased along with incremental addition of inoculum MOI (multiplicity of infection) of the virus. The promotion of STAT3 degradation also occurred in PRRSV-infected primary porcine pulmonary alveolar macrophages. Further studying showed that non-structural protein 5 (nsp5) of PRRSV induced the STAT3 degradation by shortening its half-life from 24 h to around 6 h. The nsp5-induced degradation of STAT3 was also in a dose-dependent manner. STAT3 signaling in the cells with nsp5 expression was significantly inhibited in comparison with cells transfected with empty vector. The results suggest that ubiquitin-proteasomal degradation was possibly responsible for the STAT3 decrease. No interaction between nsp5 and STAT3 was detected, suggesting that nsp5 indirectly induce the STAT3 degradation. These results indicate that PRRSV may evade the host innate antiviral response and thwart the development of adaptive immunity by promoting STAT3 degradation.

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Ubiquitination-dependent Degradation of CREB-binding Protein and Innate Immune Suppression by Nonstructural Protein 1 of PEDV

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Porcine epidemic diarrhea virus (PEDV) is an emerged swine disease in the US and has become endemic in most pig producing states posing significant economic concerns. We previously showed that PEDV possessed an ability to suppress innate immune signaling. To identify the viral antagonists for this suppression, we cloned all 16 nonstructural protein genes from PEDV, nsp1 through 16, and examined for their IFN inhibitory function using VSV bioassays and IFN reporter assays. Of 16 nsps, nsp1, nsp7, nsp14, and nsp15 were identified to suppress the IFN-beta, IRF3, and NF-κB activities. Nsp1 was a potent suppressor for IFN, and significantly impeded the activation of IFN-β promoter when stimulated with IPS-1 (MAVS), TRAF3, and activated IRF3. The molecular basis for IFN suppression by nsp1 was further investigated. Nsp1 was a nuclear protein, and in nsp1-expressing cells, IRF3 was normally transported to the nucleus, suggesting that the inhibition by nsp1 was a nuclear event. Subsequent studies indicated that CREB-binding protein in the nucleus was degraded by nsp1, which would result in the inhibition of enhanceosome assembly leading to the suppression of IFN production. The CREB-binding protein degradation by nsp1 was restored by MG132, suggesting that the degradation was proteasome-dependent. Subsequent studies showed that the CREB-binding protein was indeed ubiquitinated in nsp1-expressing cells. Our study show that PEDV inhibits the type I IFN induction, and at least one mechanism for PEDV innate immune modulation is the induction of CREB-binding protein ubiquitination and degradation mediated by nsp1.

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Porcine circovirus 2-induced endoplasmic reticulum stress, autophagy and apoptosis: independent or interrelated?

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Introduction

Porcine circovirus type 2 (PCV2) is the causative agent of the postweaning multisystemic wasting syndrome (PMWS) characterized by emaciation and lymphoid depletion. Apoptotic cell death could be one of the mechanisms of PCV2 infection. Our early studies have indicated that PCV2 induces autophagy that in turn enhances viral replication. Autophagy and apoptosis have been shown to connect with ER stress. A host of RNA viruses could differentially activate ER stress-related signaling pathways. However, there is paucity of information on the involvement of DNA viruses in ER stress. It remains unknown if PCV2 induces ER stress and if autophagy or apoptosis is primary to PCV2 infection or secondary cellular responses following ER stress.

Materials and Methods

The PK-15 cells were maintained in complete MEM containing 10% FBS. PCV2 genotype 2b strain was isolated from a diseased pig. Western blotting, confocal imaging and co-immunoprecipitation were used to probe major molecules related to ER stress, autophagy and apoptosis using specific antibodies on PK-15 cells infected with PCV2. Specific inhibitors for molecules involved in ER stress or autophagy as well as siRNA for relevant key molecules were used to examine the mechanisms of different host cell responses during viral infection.

Results

PCV2 was initially found to induce autophagy through AMPK activation via the AMPK/ERK/TSC2/mTOR pathway. Here we further show that PCV2 infection could increase the cytosolic Ca^{2+} level, which in turn activated CaMKKbeta and downstream molecules such as AMPK and CaMK1/WIPI1. Therefore, PCV2 induces autophagy through increased cytosolic Ca^{2+} and CaMKKbeta activation. We also found that PCV2 infection caused selective activation of PERK without concomitant activation of the IRE1 or ATF6 pathway. Increased expression was seen

with ATF4, which in turn induced expression of the transcription factor CHOP(C/EBP homologous protein). Both ATF4 and CHOP were induced at a later time point than PERK when the viral capsid protein expression was significantly elevated and remained high. It is clear that persistent PCV2 infection could lead to selective activation of the PERK/eIF2alpha/ATF4/CHOP axis. Because CHOP is found to activate PUMA (p53 upregulated modulator of apoptosis), which is involved in p53-dependent and –independent apoptosis induced by a variety of signals including virus infections. We postulate that PCV2-infected cells are destined to undergo apoptosis. Since inhibition of IP3R (inositol trisphosphate receptor, IP3 activated Ca^{2+} channel) did not alter eIF2alpha phosphorylation but reduced PCV2-induced autophagy, ER stress and autophagy may be independent responses during PCV2 infection. Additionally, we discovered that PCV2 hijacked autophagic and unfolded protein responses for enhancement of its replication.

Conclusions

Our findings in this report and earlier publications provide clear evidence that PCV2, though small and simple, could induce multifaceted host cell responses of ER stress, autophagy and apoptosis. Warranted for further research is to determine how these cellular events are delicately regulated during PCV2 infection. Such work may lead to better understanding of PCV2 pathogenesis.

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Efficacy of a PRRSV MLV vaccine against a genetically diverse range of PRRSV isolates

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Modified live virus (MLV) vaccines are widely used in PRRSV control strategies, where they have been shown to reduce clinical signs and production losses. PRRSV is characterized by a high level of genetic diversity, and there is no simple relationship between genetic similarity and cross-protection. This abstract summarizes the available cross-protection data for a MLV vaccine (Fostera® PRRS) based on strain P129 (lineage 8). Data are summarized for 6 experimental challenge studies, each using a different challenge strain. All are further described in peer-reviewed publications or conference abstracts, although some additional details are included here. Information on the challenge viruses is presented in Table 1. Isolates 1 and 2 were from the USA, 3 from Canada, 4 from Thailand and 5 and 6 from Korea. Several trials have been conducted using NADC20 and study 1 was selected based on protocol similarity to the other studies shown. All studies describe respiratory challenges in growing pigs vaccinated at 3 to 4 weeks of age. Challenge was 3 (study 3), 4 (studies 2 & 4), 5 (studies 5 & 6) or 24 (study 1) weeks later. There are between-study differences in the details of the experimental procedures, but post-challenge lung lesion scores from control and vaccinated groups are reported for all studies and viremia for four studies.

Comparative lung lesion scores and viremia post challenge by study are summarized in Table 2. Because of the trial design study 2 was not subject to statistical analysis. All other differences (except study 3 lung scores where p=0.071) were significant at the 0.05 level or better. The HP-PRRS isolate in study 4 was highly pathogenic, with only 20% of control pigs surviving to the planned slaughter date, compared to 80% of vaccinates.

The studies differed in important aspects and comparisons between studies are not recommended. However, within-study comparisons are valid and demonstrate significant protection across a highly diverse range of PRRSV isolates, including strains currently circulating in North America as well as Highly Pathogenic PRRS from Asia and European (Genotype 1) strains.

Table1. Challenge strains for each challenge study

Study	Isolate	Lineage	RFLP	Genetic Distance
1[1]	NADC 20	8/9	1-4-2	3.8%
2[1]	ISU-12-39404	9	1-4-2	7.8%
3[2]	FMV12-1425619	1	1-8-4	15.4%*
4[3]	10PLI(HP-PRRS)	8	NA	NA
5[4]	SNUVR090851	1	1-6-4	13.4%
6[5]	SNUVR090485	NA†	NA	35.5%

* amino acid comparison. NA=Not Available.
† Genotype 1, subtype 1 (European PRRS)

1. O'Brien B: 2015, Proc. AASV 237-238
2. Savard C et al.: 2014, Proc IPVS 307
3. Charoenchanikran P et al. 2015, CUVC
4. Park C et al: 2014, Vet Micro 172: 432-442
5. Park C et al: 2015, Vet Micro 177: 87-94

Table 2. Lung lesion scores and viremia levels

Challenge Study	Lung Score(%)		Viremia (7-13 dpc)	
	Control	Vx	Control	Vx
1	12%	1%*	NA	
2	16.9%	10.2%†	6.92	5.61†
3	11%	5%**	2.39	1.5*
4	69.9%	46.8%*	NA	
5	25.3%	13.2%*	8.1	6.8*
6	15.0%	2.5%*	4.1	0.5*

Viremia expressed as log10 of the actual viral load

*P≤ 0.05
**P≤ 0.07
†Descriptive statistics
NA=Not Available

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effects of adenoviral delivered interferon-alpha on porcine reproductive and respiratory syndrome virus infection in swine

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Type I interferons, such as interferon (IFN) alpha, contribute to innate antiviral immunity by promoting production of antiviral mediators and also play a role in the adaptive immune response. Porcine reproductive and respiratory syndrome (PRRS) has been shown to induce a meager IFN-alpha response. We administered porcine IFN-alpha using a replication-defective adenovirus vector and challenged with a moderately virulent PRRSV. There was a better clinical outcome in pigs treated with IFN-alpha, including lower febrile responses and decreased percentage of lung involvement. Viremia was delayed and there was a decrease in viral load in the sera of pigs treated with IFN-alpha. In addition, there was an increase in the number of virus-specific IFN-gamma secreting cells, as well as an altered cytokine profile in the lung 14 days post-infection, indicating that the presence of IFN-alpha at the time of infection can alter innate and adaptive immune responses to PRRSV. In a subsequent experiment we further explored the use of IFN-alpha as an adjuvant given with attenuated PRRSV virus vaccine and found that it was able to totally abolish replication of the vaccine virus resulting in no seroconversion. Current studies are examining its use as a metaphylactic treatment for an outbreak of PRRSV to both treat and prevent spread of the virus. The use of immunomodulators is a promising area for therapeutic, prophylactic, and metaphylactic use to prevent and combat infectious disease.

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Effects of Vaccination with PRRS-MLV on Post-Challenge Viremia in Young Pigs

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Introduction: The purpose of this study was to evaluate the effect of an experimental serial of a modified live PRRS virus (Formulated in accordance with the outline of production for Fostera® PRRS) on the reduction of post challenge viremia when piglets were vaccinated at 3 weeks of age and challenged at 7 weeks of age with the virulent PRRSV isolate NADC20.

Materials/Methods: Twenty (20) healthy, 3 week old piglets were vaccinated IM with an experimental serial of PRRSV-MLV (T02). A second group of 20 piglets was inoculated with 2 mL of PBS (T01). Treatment groups were housed in separate rooms during the vaccination phase. Prior to the challenge phase pigs were rehoused into one room. Pigs were challenged intranasally and intramuscularly with PRRSV NADC20 (3.0 log_{10} $TCID_{50}$ per dose). Serum samples were collected 3 times each week post challenge to detect PRRS virus (VI on PAM cells). The study concluded when ≥80% of the controls were determined to be virus negative (Day 55 post vaccination, day 27 post challenge). Duration of viremia post-challenge was determined for each animal as {[(last day of virus detected + ½ the number of study days to the next sampling day) – (first day of virus detected - ½ the number of study days to the previous sampling day)] + 1} if the animal had virus isolated, or zero (0) for animals that did not have virus isolated.

Results: The primary variable in determining reduction of post challenge viremia was duration of viremia in T02 vaccinates versus T01 controls. Duration of viremia was significantly lower (P = 0.0327) for T02 pigs versus T01 pigs. In addition, post challenge VI titers were significantly lower (P ≤ 0.0053) in T02 vaccinates versus T01 controls for Day 36 through Day 50.

Conclusions: Use of PRRSV-MLV at 3 weeks of age significantly reduced both the level and duration of viremia in piglets challenged at 7 weeks of age with a virulent PRRSV isolate. Reducing the level and duration of viremia resulted in a reduction over time of positive pigs in the vaccinated group.

<u>Implication</u>: Vaccination of a herd prior to exposure to field strains can reduce numbers of positive pigs and lower levels of viremia in a herd and result in decreased spread of infectious virus.

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Dynamic Distribution of Porcine Reproductive and Respiratory syndrome Virus (PRRSV), Porcine Circovirus type 2 (PCV2), and Mycoplasma hyopneumoniae (Mhp) in Offsprings after Using Attenuated PRRSV Vaccine in Field Sows

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Specific objective: Porcine reproductive and respiratory syndrome virus (PRRSV) is one of most important pathogens associated with porcine respiratory disease complex (PRDC) in Taiwan pig farms. The aim of this study was to compare the PRRSV-specific antibodies and pathogenic loads in serum of pigs born from sows with high (S/P ratio: 1.51-3.25) or low antibodies (S/P ratio: 0.41-1.17) against PRRSV and with immunization of PRRS modified live vaccine (Ingelvac PRRS® MLV).

Methods: Totally, 60 sows were evenly distributed into four groups: high antibodies and low antibodies against PRRSV (30 sows each). Each group was then further divided by half, where 15 sows were immunized with vaccine (Ingelvac PRRS® MLV) between 76 – 84 days of gestation (HV/LV), and the other half acted as control (HC/LC). Then, 80 4 week-old pigs randomly selected from each group were used and blood samples were collected at 4, 6, 8, 10, 12 week-old. Quantitative real-time polymerase chain reaction (qrt-PCR) was employed to quantify the levels of PRRSV, porcine circovirus type 2 (PCV2) and *Mycoplasma hyopneumoniae* (MHYO), and Enzyme-linked immunosorbent assay (ELISA) to detect the antibodies against those three pathogens.

Results and conclusions: The result showed that the antibody performances in pigs of both H-Con and H-V groups were better than the others in 4-, 6- and 8- week-old piglets. In 6-week-old pigs, the viral loads of PRRSV in group H-Con and H-V were lower than others ($p \leqq 0.05$), whereas there was no significant difference in pathogenic loads of PCV2 and MHYO among groups. Taken together, sows with high titers of PRRS antibodies might provide better protection for their offsprings during the postweaning stage.

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Immunogenicity of Mosaic DNA Vaccines against Porcine Reproductive and Respiratory Syndrome Virus (PRRSV): Induction of Antibodies and Interferon-gamma Expression in Pigs

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Objectives: Design and construct T cell epitope mosaic DNA vaccines to improve the breadth and depth of protection towards genetically and antigenically diverse PRRSV strains. Test vaccine-induced immune responses using genetically divergent virus strains.

Methods: Two DNA vaccine candidates that encode mosaic T cell epitopes derived from 748 genotype II ORF5 sequences encoding for GP5 protein were constructed. The mosaic DNA vaccines were tested in pigs in pilot vaccination/challenge trials. Gene gun, electroporation and liposomes were utilized as delivery systems in these trials. Antibody responses were monitored in vaccinated animals by indirect-ELISA. The expression of interferon-gamma and other cytokines mRNA was measured in virus-stimulated PBMCs by real-time PCR using the delta-delta method.

Results: Mosaic vaccines were shown to be functional. The levels of virus-specific antibodies detected in positive control and mosaic-vaccinated animals were generally higher than those of control animals at different time points after vaccination ($p<0.05$). There were significant differences between mosaic-vaccinated and control animals in the expression of interferon-gamma mRNA by virus-stimulated PBMCs at 21, 35 and 48 day post vaccination in Trial 3 ($p<0.05$). Expression levels of mRNA of other cytokines are being investigated. Induction of cell-mediated responses in PBMCs by divergent strains and virus neutralization assays are under investigation

Conclusions: The data shows that vaccination induced both humoral and cellular immune responses in both positive control and mosaic-vaccinated pigs but not in control animals, confirming their immunogenicity. No adverse reactions were observed upon vaccination.

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Attenuation of porcine reproductive and respiratory syndrome virus (PRRSV) by inactivating the ribosomal frameshifting products nsp2TF and nsp2N: Implication for the rational design of vaccines

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PRRSV nonstructural proteins, nsp2TF and nsp2N, were recently identified to be expressed by ribosomal frameshifting during translation of the nsp2-coding region. The nsp2, nsp2TF and nsp2N share the N-terminus PLP2 domain that was previously identified to possess innate immune suppression function. In this study, two recombinant viruses, KO1 and KO2, were further analyzed. KO1 was generated by partial inactivation of nsp2TF expression, while for KO2, the expression of both nsp2TF and nsp2N were knocked out. A multiplexed digital mRNA profiling array assay was employed to detect the expression of 579 immune genes in MARC-145 cells infected with wild-type (WT) virus, KO1 or KO2 mutants. In comparison to that of WT virus, a total of 12 and 88 differentially expressed immune genes were upregulated in cells infected with KO1 and KO2, respectively. KO2 mutant showed significant increased level of expression for certain immune genes (2 to 223-fold increase in expression compared to that caused by WT virus). Major groups of upregulated immune genes involved in cytokine-cytokine receptor interaction, antigen processing and presentation, and TLR, RIG-I and JAK-STAT pathways were identified. Increased levels of mRNA expression for type 1 IFN-a, IFN-b, and IFN-stimulated genes of GBP1, BST2, IFITM1, IFI35, IFIH1, IFIT2 and Mx1 were detected. The upregulation of 15 representative genes was validated by quantitative RT-PCR using samples from WT virus and mutants-infected MARC-145 cells, as well as in lung tissues from WT virus or mutants-infected pigs. Upregulated immune gene expression levels were correlated with increased NK cell activity and T-helper cell response in mutants-infected pigs. Furthermore, vaccination with KO1 and KO2 mutants enhanced homologous protection against a challenge infection, as was evident from reduced lung lesions and viral loads. Our data strongly implicate PRRSV nsp2TF/nsp2N in viral immune evasion and demonstrate that nsp2TF/nsp2N-deficient viruses are capable of generating protective immune responses. Thus, manipulation of nsp2TF/nsp2N expression could be used in the rational design of improved PRRSV vaccines.

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Development of a Novel Vaccine for Porcine Epidemic Diarrhea Virus

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Infections with Porcine Epidemic Diarrhea Virus (PEDV) have caused more than eight million deaths in piglets throughout North America and have resulted in significant economic losses to the swine industry. Here we describe the development of a novel inactivated vaccine for PEDV that has proven highly effective in newborn piglets. When administered to sows four and two weeks prior to farrowing, the vaccine induced high levels of antigen-specific SIgA-antibodies in colostrum and milk. High levels of neutralizing antibodies were found in serum of piglets born to vaccinated sows. Piglets were challenged at day 5 with PEDV isolate CO 025 provided by Dr. Sabrina Svenson, NVS at USDA. It was found that 95% of all piglets from vaccinated sows (n = 83) survived the infection and showed significantly reduced clinical symptoms, weight loss and viral shedding. In contrast, all piglets from unvaccinated sows displayed severe clinical symptoms including severe weight loss and dehydration, and 50% of these piglets died within 6 days post infection. These results show that this experimental vaccine is highly effective against the neonatal form of this disease. More than 10,000 doses have been produced and field trials are planned to further evaluate the vaccine in commercial settings.

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Predicting relatedness of PRRSv strains based on whole genome T cell epitope content

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We have developed an immunoinformatics tool to identify the best PRRSv vaccine to use for herd-specific PRRSv outbreaks. PRRSv (Porcine Reproductive and Respiratory virus) is an enormous economic burden to pork producers. Like many RNA viruses, PRRSv has considerable genetic and antigenic variability that has made the disease difficult to prevent with standard vaccines and an efficacious, broadly cross-protective formulation has yet to be developed. While methods for comparing existing vaccines to PRRSv strains have been informative, the 'whole gene' approach fails to estimate cross-reactivity because it does not consider the T cell epitopes that are presented to the immune system, and whether they are conserved between the vaccine and the challenge strain. For that reason, we developed an Epitope Content Comparison (EpiCC) tool to better define the degree of conservation between PRRSv vaccines and circulating strains. We propose to use this tool to identify the best vaccine to use for herd-specific PRRSv outbreaks.

We have previously developed a set of Swine Leukocyte Antigen (SLA)-restricted epitope prediction tools (PigMatrix). We further modified this tool to define relatedness based on T cell epitope content. Using this new tool (EpiCC) we screened complete genomes from 20 PRRSv and three modified live virus (MLV) vaccines. We identified epitopes predicted to bind to common class I and class II SLA alleles. Epitopes were compared and an epitope-based relatedness score (EpiCC score) was calculated. A distance EpiCC score matrix was constructed and used to built an 'epi-phylogenetic tree' that depicts the relatedness between strains based on epitope content. We observed epitope content variability across proteins and strains and differences between the whole-genome phylogeny and the EpiCC-based tree.

EpiCC provides an objective approach to aid pork producers in vaccine selection when a PRRSv strain is introduced into a herd, and to select viral epitopes for incorporation into a MLV vaccine.

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Analysis by ELISA after vaccination with an inactivated EU-typed PRRS vaccine in negative animals in a Korean farm

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Introduction: Porcine reproductive and respiratory syndrome (PRRS) causes reproductive failure and respiratory tract disease. The objective of this study was to assess the ELISA response after a sow vaccination with an inactivated EU-typed PRRS vaccine and the transfer of ELISA antibodies to their progeny, in field Korean conditions.

Materials and methods: The study was carried out in an EU-typed PRRSV negative 600-sow farm located in Boryeong city South-Korea. Eight sows randomly chosen were vaccinated (V) with PROGRESSIS® (Merial, Lyon, France) 9 weeks before farrowing and revaccinated 3 weeks later. As a non-vaccinated control group (NV), 8 other sows were injected with saline according to the same schedule. From each of the 16 litters, 5 piglets per sow were selected to be SN tested. All sows were bled on day D-63, D-42, D0 (farrowing day) and D26, and 5 of their newborn piglets were bled on day D7, D14 and D26 after birth. Antibody titers of all sera were analyzed using an indirect ELISA[1, 2] (IDEXX Laboratories. Inc., Westbrook, USA). T-test and Mann-Whitney U test of SPSS statistics 21 (IBM Corp., USA) were used for statistical significance.

Results: The results of antibody titers of sows are shown in Table 1 and that of piglets are shown in Table 2.

Table 1. Result of average PRRSV-specific antibody titers of sows and piglets by ELISA

Sows	Day -63[a]	Day -42	Day 0	Day 26
V	0.12[b] (±0.08[c])	0.67 (±0.46)	0.81 (±0.56)	0.75 (±0.52)
C	0.16 (±0.12)	0.17 (±0.12)	0.17 (±0.12)	0.19 (±0.13)
P value	0.382	0.009	0.005	0.000

Piglets	Day 7[d]	Day 14	Day 26
V	1.08(±0.21)	1.01(±0.20)	0.60(±0.10)
C	0.20(±0.03)	0.22(±0.04)	0.19(±0.04)
P value	0.000	0.000	0.000

a: Time of sows' blood sampling (1st vaccination, 2nd vaccination, parturition and weaning).

b: Average S/P ratio of each group.

c: Confidence interval(CI) in confidence level(CL) of 95%.

d: Time of piglets' blood sampling (1-wk-old, 2-week-old and weaning).

Discussion and conclusion: ELISA titers of vaccinated sows significantly increased although the ones of the control sows remained low (indicating no field contamination during the observation period). ELISA titers of piglets of the vaccinated group were significantly higher than those of the control group at every sampling point and decreased over time as expected. In this study, pre-farrowing sow vaccination with PROGRESSIS was shown to induce high level of ELISA antibodies in PRRS negative sows, that are well transferred to their piglets.

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Sows and maternal antibody analysis by SN test after vaccination with an inactivated EU-typed PRRS vaccine under Korean condition

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Introduction: Porcine reproductive and respiratory syndrome virus (PRRSV) causes reproductive failure and respiratory disease. In general, most neonatal infections can be prevented by the right passive colostrum and lactogenic immunity from the sow. The aim of this study was to assess the sow sero-neutralizing response and the

antibody transfer to piglets after sow vaccination with an inactivated EU-typed PRRS vaccine, in Korean field conditions.

Materials and methods: The study was carried out in a 600-sow EU-typed PRRSV negative farm located in Boryeong city, South Korea. Eight sows randomly chosen were vaccinated (V) with PROGRESSIS® (Merial, Lyon, France) 9 weeks before farrowing and revaccinated 3 weeks later. As a control group (NV), 8 other sows were injected with saline according to the same schedule. From each of the 16 litters, 5 piglets per sow were selected to be SN tested. All sows were bled on day D-63, D-42, D (farrowing day) and D26, and 5 of their newborn piglets were bled on day D7, D14 and D26 after birth. The Lelystad PRRSV strain and MARC-145 monolayer cells cultures were used to run the SN test[1,2]. Mann-Whitney U test of SPSS statistics 21 (IBM Corp., USA) was used for statistical significance.

Results

Table 1. Results of average PRRSV-specific antibody titres of the sows and piglets by SN test.

Sows	Day -63[a]	Day -42	Day 0	Day 26
V	0[b] (±0[c])	1.13 (±0.81)	2.63 (±0.49)	1.88 (±0.81)
C	0.25 (±0.46)	0 (±0)	0.25 (±0.46)	0 (±0)
P value	0.167	0.105	0.001	0.010
Piglet	Day 7[a]	Day 14	Day 26	
V	2.62[b](±0.26[c])	1.59(±0.36)	0.32(±0.24)	
C	0.32(±0.24)	0(±0)	0(±0)	
P value	0.000	0.000	0.011	

a: Day of sow blood sampling (1st vaccination, 2nd vaccination, farrowing and weaning).

b: Average $\log_2$ neutralizing antibody titre of each group.

c: Confidence interval(CI) in confidence level(CL) of 95%.

d: Day of piglet blood sampling(1-wk-old, 2-wk-old and weaning).

Discussion: SN titres of NV sows remain negative shows that there was not any contamination during the trial period. The SN titres of V sows clearly increased after two shots of vaccine and were significantly higher (D0, D26). The SN titres of piglets of the V group were significantly higher from control group at all stage of this study. It suggests maternal SN PRRS antibodies from sows were well intaken by their piglets. These results show that sow vaccination with PROGRESSIS improves the sows SN humoral immunity and can help to prevent infections during pregnancy and during the suckling period in baby piglets.

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Phenotypic and genotypic characterization associated with cell adaptation of porcine epidemic diarrhea virus

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In late 2013, severe large-scale outbreaks of porcine epidemic diarrhea virus (PEDV) re-emerged in Korea and rapidly swept across the country, causing tremendous financial losses to producers and customers. Despite the availability of PEDV vaccines in the domestic market, their protective efficacy in the field is still being debated. The unsatisfied effectiveness of current vaccines appears to be due to antigenic and genetic differences between vaccine

and field epidemic strains. Therefore, cell culture isolation of epidemic PEDV prevalent in the field is urgently needed to develop next generation vaccines. In the present study, one Korean PEDV strain, KOR/KNU-141112/2014, was successfully isolated and serially propagated in Vero cells for over 60 passages. The *in vitro* and *in vivo* characteristics of the Korean PEDV isolate were investigated. Virus production in cell culture was confirmed by cytopathology, immunofluorescence, and real-time RT-PCR. The infectious virus titers of the viruses during the first 60 passages ranged from 10^5 to 10^9 $TCID_{50}$ per ml. The inactivated KNU-141112 virus was found to mediate potent neutralizing antibody responses in immunized animals. KNU-141112 virus inoculation causes severe diarrhea and vomiting, fecal shedding, and acute atrophic enteritis in neonatal piglets, indicating that strain KNU-141112 is highly enteropathogenic in the natural host. In addition, the entire genomes or complete S genes of KNU-141112 viruses at selected cell culture passages were sequenced to assess the genetic stability and relatedness. Our genomic analyses indicated that the Korean isolate KNU-141112 is genetically stable during the first 60 passages in cell culture and is grouped within G2b lineage together with the recent emergent global strains.

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Genetic variations and pathogenicity analysis of a virulent PRRSV nsp2 deletion strain at different passage levels

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Porcine reproductive and respiratory syndrome virus (PRRSV) is the most common and world-widespread viral pathogen of swine. We have previously reported genomic sequences and pathogenicity of two type 2 Korean PRRSV strains belonging to the virulent lineage 1 family, which contain 3 discontinuous 111-1-19 amino acid deletions in nonstructural protein 2 (nsp2 111-1-19 DEL) compared to VR-2332. In the present study, a virulent type 2 Korean PRRSV nsp2 111-1-19 DEL strain, CA-2, was serially propagated in MARC-145 cells for up to 100 passages (CA-2-P100). To evaluate the *in vitro* immunity between parental CA-2 and cell-adapted CA-2-P100 viruses, we sought to explore alteration of inflammatory cytokine and chemokine expression in cultured porcine alveolar macrophage (PAM) cells infected with each virus using quantitative real-time RT-PCR. The expression levels of TNF-α and MCP-1 were significantly down-regulated in PAM cells during the course of CA-2-P100 infection. Animal inoculation studies were conducted to comparatively analyze the pathogenicity between parental and high-passage CA-2 viruses. The results demonstrated that the virulence of CA-2-P100 was decreased showing normal weight gain, body temperatures, and lung lesions comparable to control. Furthermore, cell-adapted CA-2-P100 infection resulted in declined and transient viremia kinetics, as well as delayed and low PRRSV-specific antibody responses in pigs. In addition, we determined the whole genome sequences of low to high-passage derivatives of CA-2. The nsp2 111-1-19 DEL pattern was conserved for 100 passages, whereas no other deletions or insertions arose during the cell adaptation process. However, CA-2-P100 contained 54 random nucleotide mutations that resulted in 25 aa changes throughout the genome, suggesting that these genetic drifts provide a possible molecular basis correlated with the cell-adapted characteristics *in vitro* and the attenuated phenotype *in vivo*. Altogether, our data indicate that the cell-attenuated CA-2-P100 strain is a promising candidate for developing a safe and effective live PRRSV vaccine.

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Isolate PEDV broadly neutralizing antibodies through single porcine B cell PCR

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<u>Introduction:</u> single B-cell PCR antibody technology is currently the most advanced antibody technology. The main principle is to sort the targeted subpopulation B cells by flow cytometry to 96-well plate, amplify and

clone antibody gene from a single B cell by PCR. This method preserves the natural pairing of light and heavy chain of antibody variable region, has advantages of genetic diversity, high efficiency, all-natural. The technology has been successfully applied to isolate broadly neutralizing antibodies against several major human RNA viruses like HIV-1 and MERS, but it has not yet been applied in the veterinary field. Porcine epidemic diarrhea virus (PEDV) is economically important swine enteropathogenic coronaviruses, causing vomiting, watery diarrhea, dehydration and high mortality rate of piglets. Despite several commercial vaccines available in the market, the vaccine efficacy is not ideal, there is urgent need to further develop next generation of highly effective vaccines and therapeutic antibodies. In this study, a single porcine B cell PCR technology has developed to isolated broadly neutralizing anti-PEDV antibodies, providing a important technology platform to isolate porcine neutralizing monoclonal antibodies and study humoral immunity.

Methods: Porcine lymphocytes from immunized pigs were sorted into 96-well PCR plate. VH and VL genes were amplified by single cell RT-PCR and cloned into pCDNA3.1 expression vector containing porcine heavy or light chain constant region after sequencing. The purified antibody was characterized by ELISA and neutralizing assay.

Results: The three commercial, cross-bred pigs with hyperimmune neutralizing titer from 1280 to 2180 IC50 were induced through prime/boost immunization. We developed a single B cell PCR antibody method. Using this method, we isolated 6 monoclonal antibodies from sorted single porcine B cells. One of them marked as pC10 targeting PEDV spike protein was able to neutralize both genotypes (classic strain G1 CV777 and field strain G2 Lnct2a).

Conclusion: A highly throughout single porcine B cell PCR method was established to isolate broadly neutralizing porcine monoclonal antibody.

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Evaluation of immunogenicity and safety of a live-vectored multi-antigen vaccine for the African Swine Fever Virus

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The African Swine Fever Virus (ASFV) causes a highly contagious fatal hemorrhagic disease in domestic swine and at present there is no treatment or vaccine available. Development of a vaccine is feasible since pigs that recover from infection with ASFV mutants are protected. However, attenuated ASFV is not a good vaccine since vaccinated pigs become life-long carriers of a mutant virus that is likely to acquire virulent traits. Previous vaccination studies suggest that induction of ASFV-specific cytotoxic T lymphocytes (CTLs) could be the key to complete protection. Hence, generation of an efficacious subunit ASFV vaccine depends on successful identification of CTL targets and a suitable delivery platform that will prime and expand lytic T-cells capable of eliminating ASFV-infected host cells and confer long-term memory. Current data suggests that subunit vaccines based on one to three of the currently defined ASFV antigens are unlikely to induce protective immunity. Thus, we developed a novel live-vectored multi-antigen vaccine formulation and conducted immunogenicity studies in commercial pigs. Prime-boost immunization with a cocktail of adenoviruses expressing multiple ASFV antigens rapidly induced unprecedented ASFV-specific IgG, IFN-gamma secreting T cell, and cytotoxic T lymphocyte (CTL) responses. The primed antibody responses underwent rapid isotype-switching within one week. Antigen-specific IgG responses increased significantly over a two-month period and underwent rapid recall upon boost four months post-priming. Post-boost titers were as high as 1: 8 x10^6 for a few animals against some antigens. In addition, all the vaccinees responded to all the antigens in the cocktail. Importantly, analysis of sera by IFA and Western blot using ASFV (Georgia 2007/1 isolate) - infected cells showed that the primed antibodies strongly recognized the actual ASF virus. Significant antigen-specific IFN-gamma responses were detected in peripheral blood mononuclear cells (PBMCs) of all vaccinees post-priming, post-boosting and in splenocytes after termination of the study. Critical for vaccine protection, robust ASFV antigen-

specific CTL responses were also detected in PBMCs of vaccinees. Taken together, the outcomes from this study showed that the adenovirus-vectored ASFV multi-antigen vaccine cocktail is capable of safely inducing strong CTL, antibody, and IFN-gamma secreting T-cell responses in commercial piglets. These findings support use of the replication-incompetent adenovirus as a vector for the development of a commercial vaccine for protection of pigs against African swine fever virus. The ability of the induced immune response to confer protection needs to be tested in a challenge study.

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Characterization of High Passages of an Interferon-inducing PRRSV Strain

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Type I interferons (IFNs), such as IFN-alpha and IFN-beta, are critical to innate immunity against viruses and play an important role in activation of adaptive immune response. An IFN-inducing PRRSV strain, A2MC2, was discovered and moderate virulence was observed in infected piglets. The objective of this study was to attenuate it by serial passaging in MARC-145 cells. We hypothesized that the serial passaging of A2MC2 would reduce or minimize previously observed mild virulence in pigs while retaining its feature of IFN induction. Here, we report that the interferon induction feature is sustained after the virus was propagated for 90 serial passages. The presence of interferons in the virus samples was monitored by a bioassay in Vero cells using NDV-GFP as an indicator virus as it is sensitive to interferons. Pretreatment of the Vero cells with the A2MC2 culture supernatant made the cells become resistant to the NDV infection. The bioassay results showed that the virus kept its capacity in inducing IFNs. The stability of this IFN induction during the virus passaging suggests that the viral genomic feature activating IFN synthesis was well preserved. The high passage virus was found to replicate faster with a higher yield and bigger plaques than the wild type virus. Infection of primary porcine pulmonary alveolar macrophages (PAMs) also led to induction of interferons. Sequencing analysis indicated that the A2MC2 P90 had an identity of 99.8% to the wild type virus on nucleotide basis. The A2MC2-P90 genome has a deletion of 543 nucleotides (nt) in ORF1a in comparison with wild type A2MC2, leading to a deletion of 181 amino acids (aa) in nsp2. Moreover, compared to the wild type, the A2MC2 P90 has 35 nt and 26 aa differences, throughout the genome. These mutations appear to have minimum effect on the feature of IFN induction, as P90 also induces IFN production in infected cells. Interestingly, the sequencing results show that 14 of the 15 nt conserved in wild type A2MC2 genome in comparison to VR-2332 and MLV remain unchanged in the P90 virus genome. Similarly, 5 of the 6 unique aa in the wild type A2MC2 were conserved in the P90. These results suggest that A2MC2 can be further explored for development of an improved vaccine against PRRS.

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African swine fever virus serotype-specific antigens CD2v and C-type lectin are necessary for protection against African swine fever

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African swine fever (ASF) is arguably the most significant emerging disease threat for the swine industry worldwide. Devastating ASF outbreaks and the continuing epidemic in Russia (2007 – to date) and Eastern Europe (2014 - to date) underscore the significance of this disease. There is no ASF vaccine available; however, it is clear

that vaccination is possible since protection against homologous reinfection has been definitively demonstrated. Available data from vaccination/challenge experiments in pigs suggest that ASF protective immunity is hemadsorption inhibition (HAI) serotype-specific. A safe and efficacious DIVA (Differentiate Infected from Vaccinated Animal) compatible ASFV vaccine would provide a critical tool for emergency disease response and control and reduce the risk for pork producers.

Better understandings of ASFV HAI serologic group diversity in nature as well as identification of serotype-specific antigens (SSAs) involved in protective immunity are needed for eventual vaccine design and development. Recently, we have shown that two ASFV proteins, CD2v (EP402R) and C-type lectin (EP153R), are necessary and sufficient for mediating HAI serologic specificity (J. Gen. Virol. 96:866-873).

Here, using ASFV inter-serotypic chimeric and/or gene-deleted ASF viruses and vaccination/challenge experiments in pigs we demonstrate that serotype-specific CD2v and C-type lectin proteins are necessary for generation of homologous protective immunity in pigs. Thus, CD2v and C-type lectin proteins represent significant ASFV SSAs that should be targeted in vaccine design and development. Additionally, data are consistent with and support the emerging concept that ASF protective immunity is HAI serotype-specific.

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Impact of Fostera® PRRS Vaccination on Lineage 1 PRRSV Challenge

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This study evaluated the efficacy of Fostera PRRS vaccine in pigs challenged with a 2014 heterologous lineage 1 field PRRS virus.

Materials and Methods: A field virus, ISU 14-4099, was selected and found to be 84.4% identical to Fostera PRRS vaccine virus at the ORF 5 nucleotide level and to cluster with lineage 1 reference viruses. The virus had a RFLP cut pattern of 1-18-2. Forty eight three-week-old mixed breed castrated male swine were sorted by weight and randomized into two experimental groups: vaccinated and challenged (V/C) and non-vaccinated and challenged (NV/C). V/C pigs were administered one 2 mL dose of Fostera PRRS vaccine intramuscularly at 4 weeks of age (study day 0 dpv) and the NV/C pigs were administered one 2 mL dose of sterile saline intramuscularly at the same time. 10 non-vaccinated/non-challenged (NV/NC) pigs were maintained in a separate room to serve as negative controls. All groups were maintained in separate rooms for the vaccination phase. On study day 26, half of the pens of pigs from the NV/C and the V/C rooms were swapped between the two rooms. On study day 28 (0 dpi), all pigs in the NV/C and V/C groups were challenged with one 2 mL dose of isolate ISU-14-4099 at 10^5 $TCID_{50}$/mL by the intranasal route and one 2 mL dose of the same challenge virus intramuscularly. Pigs in the NV/NC group were inoculated similarly using sterile cell culture media. Pigs were necropsied on 12 dpi and the percentage of the surface area of each lung lobe affected with pneumonia was visually estimated by a single observer blinded to treatment group. Five sections of each lung were collected, fixed and scored for severity of interstitial pneumonia by a single histopathologist blinded to treatment group. Each section was scored on a 0-6 scale and the five scores were averaged for each lung. The pigs were weighed on days -7 dpv, -2 dpi and 12 dpi. Sera were collected at 0 dpv, 14 dpv, 3 dpi, 7 dpi, and 12 dpi. The pen was the experimental unit with two pigs per pen. Percent pneumonia was analyzed using the Wilcoxon rank-sum test and weight data was analyzed using analysis of variance. A p-value ≤ 0.05 was considered significant. Variables associated with the NC/NV group were not statistically analyzed.

Results: All pigs in the V/C group were PRRSV ELISA positive by 14 dpv. All pigs in the NV/C group were ELISA and PCR negative for PRRSV in serum at 14 dpv and 28 dpv. NV/NC pigs remained ELISA and PCR negative throughout the study. On days 3, 7 and 12 post-challenge, both V/C and NV/C groups were viremic, with virus levels significantly lower in V/C vs NV/C on all days. Mean percent pneumonia was reduced by 32% in the V/C group compared to the NV/C group (mean 15.38% vs 10.47%; p=0.1487). Microscopic lung lesion scores were not significantly lower in the V/C group compared to the NV/C group. Mean average daily gain lacked a statistical difference for the time period from -7 dpv to -2 dpi. Post-challenge (-3DPI to 12 DPI) average daily gain was significantly higher in the V/C group (.97 lbs.) compared to the NV/C group (.71 lbs.) (p=0.0112).

Discussion: This study demonstated the ability of Fostera PRRS vaccine to provide partial protection against a 2014 PRRSV lineage 1 field strain in that ADG was higher and virus titers were lower after challenge in the V/C group versus the NV/C group.

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Efficacy of Fostera® PCV MH vs Other Combination Vaccines Following Dual Challenge with Mycoplasma hyopneumoniae and PCV2

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Introduction

A research study was conducted to evaluate vaccination regimens using 1 or 2 doses of Fostera PCV MH compared to other competitive vaccines in their ability to limit M. hyo lung lesions and PCV2viremia in swine challenged with both virulent M. hyo and PCV2b.

Materials and Methods

The trial involved 248 healthy piglets (mixed gender) serologically negative for M. hyo and PCV2. Piglets were weaned at approximately 3 weeks of age (study day 0) and randomly allocated to 5 treatment groups by blocks based on body weight. The 'vaccination phase' of the study (3 to 9 weeks of age) involved administration of 1 or 2 mL of the following products: Fostera PCV MH (one 2-mL dose), n=48; Fostera PCV MH (two 1-mL doses, 2nd dose on day 14), n=48; Ingelvac® CircoFLEX-MycoFLEX® (one 2-mL dose, mixed into a single bottle before vaccination), Boehringer IngelheimVetmedica, n=48; Circumvent® PCV-M (two 2-mL doses, 2nd dose on day 21), Intervet/Merck Animal Health, n=48. The 'challenge phase' of the study (9 weeks of age and following) involved 2 separate events: M. hyo challenge: at approximately 9 weeks of age (6 weeks after initial vaccination), each pig was challenged intratracheally; PCV2b challenge: at approximately 10 weeks of age (7 weeks after initial vaccination), each pig was challenged via both the IM and intranasal routes. The primary variables of interest were the severity of M. hyo lung lesions and PCV2 viremia. Individual serum samples were collected at study days 0, 28, 41, 48, 56, 63, and 70. Samples were analyzed by quantitative polymerase chain reaction (qPCR) for detection of PCV2 viremia, and serological testing using enzyme-linked immunosorbent assay (ELISA) assessed M. hyo and PCV2 antibody titer. At necropsy, sections of 3 lymph nodes (tracheobronchial, mesenteric, inguinal) and tonsil were collected from each pig and submitted for histopathological examination for lymphoid depletion (associated with PCVAD) and histiocytic replacement, as well as testing for PCV2 antigen by immunohistochemistry (IHC). Data were statistically analyzed by appropriate methods using each pig as the experimental unit. Statistical significance recognized at $P \leq 0.05$

Results

Only 2.8% to 3.5% lesion severity was observed for pigs vaccinated with Fostera PCV MH. In contrast, pigs vaccinated with Ingelvac CircoFLEX-MycoFLEX demonstrated no significant lesion reduction compared to controls ($P > 0.05$). Furthermore, both Fostera PCV MH groups experienced significantly less M. hyo lesion pathology than Ingelvac Circo -FLEX-MycoFLEX vaccinates (69-75% reduction with Fostera PCV MH, $P \leq 0.05$). Improvements in M. hyo protection relative to controls and Ingelvac CircoFLEX-MycoFLEX were also provided by Circumvent PCV-M. All vaccinated groups significantly reduced PCV viremia incidence relative to controls (70-91% reduction, $P \leq 0.05$).

Discussion

This study further confirms that Fostera PCV MH is an effective combination vaccine that helps provide protection from both PCVAD and mycoplasmal pneumonia.

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Safety of Fostera® PCV MH vs Other Combination Vaccines

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Introduction

A research study was conducted to evaluate vaccination regimens using 1 or 2 doses of Fostera PCV MH compared to other competitive vaccines in their ability to limit reaction due to vaccination.

Materials and Methods

The trial involved 248 healthy baby piglets (mixed gender) serologically negative for M. hyo and PCV2. Piglets were weaned at approximately 3 weeks of age (study day 0) and randomly allocated to 5 treatment groups by blocks based on body weight. The vaccination involved administration of 1 or 2 mL of the following products at approximately 3 weeks of age (right neck) with a second dose administered at 5 to 6 weeks of age to 2 groups (left neck): Fostera PCV MH (one 2-mL dose), n=48; Fostera PCV MH (two 1-mL doses, 2nd dose on day 14), n=48; Ingelvac® CircoFLEX-MycoFLEX® (one 2-mL dose, mixed into a single bottle before vaccination), Boehringer Ingelheim Vetmedica, n=48; Circumvent® PCV-M (two 2-mL doses, 2nd dose on day 21), Intervet/Merck Animal Health, n=48. Pigs were observed throughout the study for general health and clinical signs of respiratory distress, lethargy, wasting, etc. The primary variable was injection site reactions. All right-neck injection sites were observed and palpated for adverse reactions on days 1, 4, and 7, and sites on both the left and right neck were assessed on days 15, 18, 22, 23, 28, and 35. Reaction severity was scored using a numerical system ranging from 0 to 3 (0=normal, 3=severe). Any injection site reactions were monitored until resolution. Data were statistically analyzed by appropriate methods using each pig as the experimental unit. Statistical significance recognized at $P \leq 0.05$

Results

With the exception of the Circumvent PCV-M group, all other vaccinates and controls had low incidences of injection reactions for both the first and second injections. However, pigs vaccinated with Circumvent PCV-M exhibited significantly more site reactions, especially when the second vaccination was administered. Notably, the incidence of second-vaccination site lesions was reduced 86.5% ($P \leq 0.0001$) for pigs vaccinated with Fostera PCV MH compared to those vaccinated with Circumvent PCV-M. The duration of site reactions was also much longer in the Circumvent PCV-M group compared to other vaccines, particularly with the second injection (5.38 days vs 0.27-0.68 days, $P \leq 0.05$). Outcomes were further confirmed by results of reaction severity scoring. Over 40% of pigs receiving the second Circumvent PCV-M vaccination demonstrated a 'severe' injection site reaction score (3: over 5-cm-diameter swelling, evidence of irritation and pain such as persistent rubbing or withdrawal and vocalization upon palpation, and/or an abscess). These various site data indicate that the Circumvent PCV-M formulation triggered much more site reactivity than other test vaccines (which were not different than saline controls).

Discussion

This study confirms that Fostera PCV MH is a safe combination vaccine with reaction rates not different than saline.

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Expression of antigenic epitopes of porcine reproductive and respiratory syndrome virus (PRRSV) in a modified live-attenuated porcine circovirus type 2 (PCV2) vaccine virus (PCV1-2a) as a potential bivalent vaccine against both PCV2 and PRRSV

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Introduction: Porcine circovirus type 2 (PCV2) is the causative agent of porcine circovirus-associated disease (PCVAD). Porcine reproductive and respiratory syndrome (PRRS) is caused by PRRS virus (PRRSV). Both PCV2 and PRRSV have caused devastating diseases in swine industry worldwide, resulting in immense economic losses. One of the most common co-infections in the swine industry is PCV2 and PRRSV. Previously, it has been demonstrated that a genetically modified infectious PCV1-2a can tolerate up to a 27 amino acid insertion in the C-terminus of ORF2 without affecting infectivity and generate a dual immune response against PCV2cap and the inserted epitope tag.

Objective: The aim of this study was 1) to generate chimeric viruses containing neutralizing epitopes of PRRSV using the backbone of the non-pathogenic PCV1-2a vaccine strain $_{(vs)}$, and 2) to evaluate infectivity and immunogenicity of PCV1-2a-PRRSV$_{EPI}$ chimeric viruses *in vivo*.

Materials and methods: Four different B-cell linear epitopes derived from PRRSV strain VR2385, including GP2 II (aa 40–51, ASPSHVGWWSFA), GP3 I (aa 61–72, QAAAEAYEPGRS), GP5 I (aa 35–46, SSSNLQLIYNLT), and GP5 IV (aa 187–200, TPVTRVSAEQWGRP), were cloned individually in frame into the C-terminus of the PCV1-2a$_{vs}$ capsid gene. *In vitro* infectivity and co-expression of the PCV2-capsid protein and PRRSV epitopes were evaluated by IFA. Infectivity and immunogenicity *in vivo* was evaluated by inoculation of a total of 21 specific-pathogen-free (SPF) pigs, randomly assigned into seven groups of three pigs each, including two positive control groups (PCV1-2a and PRRSV), a negative control (MEM-treated group), and four groups, one for each of the PCV1-2a-PRRSV$_{EPI}$ chimeric viruses. Serum samples were collected from each pig prior to inoculation and weekly thereafter for a period of 8 weeks. Laboratory procedures performed included Taqman® qPCR for quantification of viral DNA loads in sera and tissues, serological evaluation of IgG anti-PCV2 specific antibodies and anti-PRRSV$_{EPI}$ antibodies by ELISA, and serum virus neutralization assay to evaluate the neutralizing activity against PCV2 and PRRSV-VR2385.
Results: Four PCV1-2a-PRRSV$_{EPI}$ were infectious *in vitro* and co-expressed PCV2cap as well as the respective PRRSV epitopes. Animal studies showed that two PCV1-2a-PRRSV$_{EPI}$ chimeric viruses produced viremia and replicated in lung and tracheobronchial lymph nodes. Significant levels of IgG anti-PCV2 antibodies were detected from 28 dpi in PCV1-2a and PCV1-2a-PRRSV$_{EPI}$GP3I groups, 42 dpi in the PCV1-2a-PRRSV$_{EPI}$GP5IV group, all remained seropositive at the end of the study at 56 dpi. Two of the four chimeric viruses, PCV1-2a-PRRSV$_{EPI}$GP3IG and PCV1-2a-PRRSV$_{EPI}$GP5IV, elicited neutralizing antibodies against PRRSV VR2385 as well as PCV2 (strains PCV2a, PCV2b, and mPCV2b).
Conclusions: Our results demonstrated that PCV1-2a-PRRSV$_{EPI}$ chimeric viruses were infectious *in vitro* and *in vivo*. More importantly, we found that two chimeric viruses elicited neutralizing antibodies against PRRSV-VR2385. Overall, the results have important implications for exploring the potential use of PCV1-2a vaccine virus as a live virus vector to develop bivalent MLVs against both PCV2 and PRRSV.

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Evaluation of the immune response of pigs after immunization with various African Swine Fever Virus antigens

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African Swine Fever virus (ASFV) causes a severe disease of domestic pigs, but persistently infects its natural hosts (warthogs, bushpigs, and soft ticks of the *Ornithodoros* genus) without inducing clincal signs. In domestic pigs, the virus typically causes a hemorrhagic fever with high mortality rates. Previously being a disease endemic in sub-Saharan Africa, outbreaks of African Swine fever have been reported since 2007 in the Trans-Ckausasus region, in Russia, Ukraine, Poland and the Baltic states. To date, there is no effective vaccine against ASFV available.
The purpose of this study was to evaluate the immune response of pigs to various ASFV antigens including recombinant proteins and DNA vaccine constructs, using a heterologous prime-boost vaccination approach. The ASFV genes encoding the structural proteins p15, p35, p54 and CD2v (CD2-like) were synthesized based on the ASFV isolate Georgia/2007 and the respective recombinant proteins were expressed in a baculovirus expression system. The DNA vaccine constructs were cloned into pcDNA3.1 expression vector and included the p72, p32, CP312R and CD2v genes. Three-week old piglets were used for the immunogenicity study. The vaccination groups consisted of 8 groups (5 pigs/group) which were immunized with a combination of different recombinant proteins and plasmid DNAs. The piglets were inoculated intramuscularly with 100µg of recombinant protein mixed with ISA25 adjuvant and 100µg of plasmid DNA. Piglets were inoculated three times at two-week intervals and euthanized one week after the last immunization. Blood collection was carried out on the day of vaccination and at the time of euthanasia. ASFV-specific antibody responses in serum of immunized pigs were evaluated using ELISA, western blot and virus neutralization tests. The results of ELISA and western blot showed that antibodies were induced against each recombinant protein (p15, CD2v, p54 and p35). In preliminary virus neutralization assays we detected neutralizing activity mainly in sera from pigs immunized with structural ASFV proteins. These results will guide us in our efforts to

develop an ASF vaccine based on a heterologous prime-boost approach using both, recombinant protein and DNA constructs.

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Chimeric porcine reproductive and respiratory syndrome virus containing shuffled multiple envelope genes confers cross-protection in pigs

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The extensive genetic diversity of porcine reproductive and respiratory syndrome virus (PRRSV) strains is a major obstacle for vaccine development. We previously demonstrated that chimeric PRRSVs in which a single envelope gene (ORF3, ORF4, ORF5 or ORF6) was shuffled via DNA shuffling had an improved heterologous cross-neutralizing ability. In this study, we incorporate all of the individually-shuffled envelope genes together in different combinations into an infectious clone backbone of PRRSV MLV Fostera® PRRS. Five viable progeny chimeric viruses were rescued, and their growth characteristics were characterized *in vitro*. In a pilot pig study, two chimeric viruses (FV-SPDS-VR2, FV-SPDS-VR5) were found to induce cross-neutralizing antibodies against heterologous strains. A subsequent vaccination/challenge study in 72 pigs revealed that chimeric virus FV-SPDS-VR2 and parental virus conferred partial cross-protection when challenged with heterologous strains NADC20 or MN184B. The results have important implications for future development of an effective PRRSV vaccine that confers heterologous protection.

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The origin and evolution of European PRRSV Type 2 strains

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Porcine reproductive and respiratory syndrome virus (PRRSV) emerged as a swine pathogen in North America and Europe nearly simultaneously in the late 1980ties. The European and North American isolates were quickly found to be only distantly related by nucleotide sequence comparisons and were thus defined and later officially designated as Type 1 and Type 2, respectively. At present both genotypes are globally spread.

The presence of PRRSV Type 2 in Europe was reported in 1995 through the introduction into Denmark of the Ingelvac PRRS Vet vaccine (now known as Ingelvac MLV, Boehringer Ingelheim). Subsequently, this genotype has been reported sporadically in other countries, including those where Ingelvac MLV has not been licensed. Additional introductions of PRRSV Type 2, not related to the vaccine, have been documented in Hungary and in Slovakia (Balka et al., 2008; Vilcek et al., 2013). Recently Balka et al. (2015) has shown that the Hungarian isolate from 2012 (PRRSV-2/Hungary/102/2012) belongs to the lineage 1 or lineage of PRRSV Type 2. The most extensive study on PRRSV Type 2 diversity in Europe was recently published by Kvisgaard et al. (2013) who described the situation in Denmark.

In the present study we have compared about 120 ORF5 sequences of PRRSV Type 2 originating from Europe from 1996-2013. Most of the sequences originated from Denmark, Germany and the Netherlands but also from Poland, Hungary, Lithuania, Spain and Austria.

The majority of ORF5 sequences obtained in Europe belong to lineage 5.1 of Type 2 PRRS, which includes Ingelvac MLV (Shi et al., 2010). Five Hungarian and 1 Romanian sequences belong to lineage 2, a highly virulent group of viruses that originated in eastern Canada. As expected, isolates with high sequence identity (>97%) to Ingelvac MLV were found in all countries between 1996 and 2013. In addition, we found a cluster of lineage 5.1

sequences obtained after 2006 with the pairwise similarities to Ingelvac MLV as low as 93%. Two sequences from one German farm were clustered separately with the pairwise identity to Ingelvac MLV of only 91%.
In summary, most of the Type 2 sequences in Europe are derived from Ingelvac MLV. Shedding and spread of vaccine-related strains of either genotype in the field was previously documented. However, the lineage 1 sequences from Hungary and Romania originated from independent introduction of Type 2 strains in Europe, most likely from eastern Canada. Anecdotal information suggests a first appearance of such strains in Slovakia in the early 1990's and later spread in the region. The origin of the lineage 5.1 strains with pairwise identity to Ingelvac MLV <95%, and especially the German strain having only 91% identity, is difficult to establish. Maximum likelihood analysis and comparison to lineage 5.1 strains from North America are consistent with independent introduction of Type 2 strains in Europe or prolonged maintenance of locally evolving populations derived from Ingelvac MLV. Information on PRRSV Type 2 distribution in Europe and genetic diversity are still very limited; hence, the epidemiology of this genotype may be even more complex.

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Assessing the phylogenetic utility of PRRSV fragments to reconstruct the evolutionary history of the virus

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Currently, phylogenetic analyses based on PRRSV ORF5 are widely used in evolutionary studies and monitoring to assign PRRSV isolates to ca given clade, subgenotype and genotype. However, the fact that differential patterns of recombination, saturation, selection and mutation may influence the topologies of phylogenetic trees may create some incertitude about the precision of the classifications made based on ORF5. The objective of the present work was to evaluate the importance of the evolutionary forces cited above along the PRRSV genome, and the reliability of different fragments as phylogenetic markers at the macro- and micro-evolutionary timescale.
A dataset of 444 PRRSV genomes from genotypes 1 (54) and 2 (390) have been analyzed to evaluate the phylogenetic utility of the non-structural proteins (nsp) and the structural ones. Every sequence was firstlyidentified with the accession number, the isolate name, the country of origin, the collection date and the pathogenicity (when known). Saturation and recombination analyses were carried out by DAMBE 5.6.9 for windows and GARD (www.datamonkey.org), respectively. Then, datasets were purged to remove saturated positions and recombinant sequences. Phylogenetic reconstructions (MEGA 6.0), selection profiles (SNAP; http://www.hiv.lanl.gov/content/sequence/SNAP/SNAP.html) and rates of substitution (BEAST v2.3.0) were estimated for every fragment.
The analyses identified at least seven recombination breakpoints along PRRSV genomes in both genotypes. Accordingly, significant conflicting topologic clustering (Kishino-Hasegawa tests) were reported among different genome fragments. Interestingly, preliminary results point to potential recombination between European and Asian strains within genotype 1. Moreover, substantial levels of saturation were detected in the third position of nsp1, 2 and 4 for both genotypes and in nsp7 and 11 for genotype 1, indicating that the poor phylogenetic utility of those fragments. Also, selection analysis showed that differential rates shape the PRRSV genome. The results obtained in the present work show that evolutionary forces have a differential impact along PRRSV genome determining the phylogenetic utility of every viral protein. So, the presence of multiple recombinant strains strengthens the need of full genome sequence analysis in PRRSV to adequately reflect the evolutionary history of this virus. Phylogenetic analyses based on nsp1, 2, 4 (genotype 1 and 2), 7 and 11 (genotype 1) should not consider the third codon position. Further analyses will be carried out in order to determine the use of different targets in the PRRSV genome at long- and short-term evolution.

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Porcine reproductive and respiratory syndrome virus hijacks nanotubes for intercellular spread: an alternative pathway used for nidovirus transmission

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The primary mechanism for spreading of most viruses, including porcine reproductive and respiratory syndrome virus (PRRSV) and other nidoviruses, is through the entry of cell-free virions into naïve host cells. In this study, we found an alternative pathway for PRRSV transmission, in which the virus uses intercellular nanotube connections to spread viral infectious core materials to neighboring cells. In PRRSV infected cells, nanotubes were observed connecting two distant cells with contiguous membranes under the confocal microscopy, and the core infectious viral machinery (genomic RNA and viral proteins) was observed inside the intercellular nanotube connection. A live-cell movie of PRRSV containing green fluorescent protein (GFP)-tagged nsp2 shows viral proteins moving from one cell to another through a nanotube connection. This phenomenon was also observed in cells infected with equine arteritis virus and porcine epidemic diarrhea virus, suggesting that intercellular nanotube connections serve as an alternative pathway for cell-to-cell spreading of nidoviruses. In MARC-145 cells expressing PRRSV receptor, infectious viral core materials were still detected moving from one cell to another under the condition of existing viral neutralizing antibody, while in HEK293-T cells lacking PRRSV receptor, intercellular transport of viral materials was also observed in cells transfected with PRRSV genomic RNA. The intercellular nanotube connections contain a core of filamentous actin (F-actin) with myosin associated as a molecular motor. Immunoprecipitation results showed that PRRSV nsp1β, nsp2-related proteins and GP5 can be co-precipitated with F-actin and myosin-IIA. The presence of drugs inhibiting actin polymerization or the myosin-II activation precluded the formation of nanotubes and viral clusters in PRRSV-infected cells. Similar results were also observed with several other nidoviruses. These data lead us to propose that PRRSV (and possibly other nidoviruses) hijacks cytoskeletal machineries for high-speed cell-to-cell spread and the intercellular nanotubes could be used as an alternative pathway for the virus to escape the host immune response.

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Proteolytic Processing of Porcine Reproductive and Respiratory Syndrome Virus Replicase ORF1a Polyprotein

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The porcine reproductive and respiratory syndrome virus (PRRSV) replicase open reading frame 1a (ORF1a) polyprotein is predicted to be proteolytically processed by virus-encoded proteases. In this study, the proteolytic processing products and actual cleavage sites were identified by using protein microsequencing, recombinant vaccinia virus/T7 polymerase expression system, and site-directed mutagenesis. Protein microsequencing identified that the cleavage site between nsp1alpha/1beta, nsp1beta/nsp2, and nsp2/nsp3 was located at H_{180}/S_{181}, G_{385}/A_{386}, and G_{1446}/A_{1447} of type 1 PRRSV, respectively. Transient expression of nsp2-8, nsp3-8, nsp4-8, nsp5-8 in the recombinant vaccinia virus/T7 RNA polymerase system identified cleavage products of nsp2, nsp3, nsp4, nsp7(alpha+beta) and various cleavage intermediates. The result revealed the existence of two alternative proteolytic processing pathways depending on the presence or absence of nsp2. The identity of each cleavage product was further verified by site-directed mutagenesis analysis of individual cleavage site in nsp3-8 and nsp4-8. This study constitutes the first in-depth experimental analysis of PRRSV replicase processing, and demonstrated the significant conservation of replicase protein processing scheme within the arterivirus family.

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ASFV p54 epitope mapping using polyclonal swine sera and monoclonal antibodies

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African swine fever virus (ASFV) is a large enveloped virus with icosahedral symmetry belonging to the family Asfarviridae. The genome is composed of double stranded DNA with a length ranging from 170 to 193 kbp and encodes for more than 100 polypeptides. It was shown that antibody-mediated virus neutralization mechanism is important for inducing protective immune response. The envelop protein p54 is among the most immunogenic ASFV proteins. Although anti-p54 antibodies are produced after natural infection or vaccination, there is little or no information on the immunogenic epitopes that they recognize.

The goal of this study was to identify p54 immunogenic epitopes using sera from immunized pigs and monoclonal antibodies (Mabs). The ASFV p54 protein (based on ASFV BA71V strain), from amino acid 54 to 183, was divided into 3 overlapping fragments of approximately 55 amino acids (aa) in length. Each gene sequence was commercially synthesized, cloned into TOPO vector and transformed in competent NEB10 beta cells. The fragments were then sub-cloned, downstream to a histidine-ubiquitin gene, into pHUE expression vector and transformed into BL21 (DE3) chemically competent *E.coli* cells. The recombinant proteins were expressed *in vitro*, purified and used as antigen in indirect ELISA and Western blotting (WB). The p54 fragments were tested by ELISA and WB against a panel of 12 Mabs as well as polyclonal swine sera. The Mabs were produced against ASFV Georgia/07 strain and were obtained from Plum Island. While, the polyclonal sera, originated from pigs immunized with a defective alphavirus replicon particle expressing the p54 whole protein (based on BA71V strain).

There was no difference between the sera collected at day 0 and at day 57 when tested by ELISA. However, the polyclonal sera reacted by WB to the p54 region between aa 54 and 83 (2 sera); aa 83 and 113 (4 sera); aa 143 and 183 (1 serum); aa 83 and 143 (3 sera). On the other hand, 5 Mabs reacted by ELISA and WB with the regions between aa 54 and 83 (1 Mabs), aa 83 and 113 (1 Mabs), aa 113 and 143 (2 Mabs), and aa 83 and 143 (1 Mabs). However, 7 Mabs did not react with any fragments as well as the whole protein. The latter result could be due to the fact that, at positions 80, 144, 145, and 148, there are amino acid differences between BA71V and Georgia/07.

Overall, the Mabs and the pig sera recognized overlapping regions. This is the first report describing epitope mapping of p54 protein. Future studies will be directed towards the fine mapping of the identified regions.

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Epitope Mapping of ASFV p72 Capsid Protein using Polyclonal Swine Sera and Monoclonal Antibodies

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African Swine Fever (ASF) is a hemorrhagic disease of domestic pigs caused by African Swine Fever Virus (ASFV), currently the only member of the family *Asfarviridae*. It is a large enveloped virus that contains a linear double-stranded DNA of 170–190 kbp, encoding more than 150 proteins. ASFV major capsid protein p72 makes up about 32% of the total protein mass of the virion, and has been used as an antigen for the development of

serological assays. So far, only one conformational neutralizing epitope has been identified on p72, and more information on the whole protein is lacking.

The objective of this study was to identify p72 antigenic regions using polyclonal swine sera and a panel of monoclonal antibodies (Mabs). The p72 protein (based on ASFV BA71V strain), from amino acid 1 to 345, was divided into 5 overlapping fragments. Fragments were produced by PCR or commercially synthesized. The nucleotide sequence of the fragments was then cloned into pHUE expression vector and transformed into BL21(DE3) *E. coli* competent cells. The recombinant proteins were expressed *in vitro*, purified and used as antigens in indirect Enzyme-Linked Immunosorbent Assay (ELISA) and Western Blotting (WB). Each p72 fragment was tested by ELISA and WB against a panel of Mabs and polyclonal swine sera. The Mabs were produced against a current ASFV strain, Georgia/07, and were obtained from Plum Island. While, the polyclonal sera were from pigs immunized with a defective alphavirus replicon particle, RP-sHA-p72, expressing a recombinant protein composed of the extracellular domain of the ASFV HA protein (based on the E75 strain) together with the whole p72 protein (based on the BA71V strain).

The polyclonal sera reacted to the p72 region between amino acids 1 and 83 and amino acids 250 and 280. While, the monoclonal antibodies tested so far reacted by ELISA and WB with the p72 regions between amino acids 100 and 171; 180 and 250; 280 and 345. The p72 from BA71V is suitable to be used as an antigen for epitope mapping of the Mabs raised against the Georgia/07 strain. In fact, the amino acid alignment between p72 from BA71V and Georgia/07 showed only one amino acid difference (at position 126) in the first 345 amino acids, and this variation did not have any effect on the reactivity of the Mabs tested. Ongoing studies are carry out to complete the testing of the p72 Mabs. Future studies aim at the fine mapping of the identified epitopes.

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Analysis of intra-host genetic diversity in Rift Valley Fever virus infection of ruminants

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Rift Valley Fever (RVF) is an arthropod-borne zoonotic disease caused by RVF virus (RVFV) of family *Bunyaviridae*, genus *Phlebovirus* that affects ruminants and humans. The tripartite viral genome is composed of large (L), medium (M) and small (S) segments of single-stranded, negative-sense RNA. The high mutation rate of RNA viruses increases the ability of the viruses to adapt to diverse selective pressures. To analyze the intra-host viral diversity, samples were collected at different time points post infection from sheep experimentally infected with RVFV strains Kenya 128b-15 and (Saudi Arabia) SA01-1322 isolated in 2006 and 2001, respectively. Full-length viral genomes were amplified by RT-PCR from the viral inocula and tissue samples (blood, liver and spleen) collected during peak viremia. For analysis of the terminal regions of the genome segments, viral RNA was circularized and the tandem region containing both the 5' and 3' ends of the viral genome was amplified by RT-PCR. The amplified products were subjected to deep sequencing on the Illumina Miseq platform. The viral sequences from the tissues were compared with viral inocula and the type of mutations along with the frequency (% of viral population) of quasispecies was analyzed. The L segment of the Kenya strain showed 4-6 synonymous mutations (5-32%), the M segment showed 2-4 non-synonymous mutations (13-99.7%) and two synonymous mutations (7-25%) within the NSm/Gn/Gc genes and one synonymous mutation in the UTR, and the S segment showed two synonymous mutations one in the NSs gene (16%) and one in the N gene (43-50%). The L segment of the SA01 strain showed two synonymous mutations (12.4-98.6%) with one in the UTR (99.8%) and one non-synonymous mutation (98.6%), the M segment showed three synonymous mutations (99%) in the UTRs, two synonymous mutations (78-99%) in the NSm gene and one non-synonymous mutation in Gn gene (7.4%); the S segment showed two synonymous

mutations (99%) one each in the NSs gene and UTR and two non-synonymous mutations (99%) in the NSs gene. In conclusion, deep sequencing provides insights into the dynamics of variants within the host but the biological significance of these mutations warrants further examination.

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Amino acid residues Ala283 and His421 in the RNA-dependent RNA polymerase of porcine reproductive and respiratory syndrome virus play important roles in viral Ribavirin sensitivity and quasispecies diversity

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The quasispecies diversity of RNA viruses is mainly determined by the fidelity of RNA-dependent RNA polymerase (RdRp) during viral RNA replication. Certain amino acid residues play an important role in determining the fidelity, and such residues can be substituted with other amino acids to produce high fidelity viral strains. In this study, under the selection of Ribavirin two amino acid substitutions (A283T, H421Y) in the RdRp of porcine reproductive and respiratory syndrome virus (PRRSV) were identified. The two substitutions were subsequently found to confer PRRSV the properties of increased Ribavirin-resistance and restricted quasispecies diversity. The results indicated that these two amino acid residues (Ala283 and His421) play a crucial role in PRRSV replication by affecting the fidelity of its RdRp. The results have important implication for understanding the molecular mechanism of PRRSV evolution and pathogenicity, and developing safer PRRSV modified live-attenuated vaccine (MLVs).

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A Novel Function of PRRSV Nsp1 for Inhibition of Host Cell mRNA Nuclear Export and Suppression of Host Protein Synthesis

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Host cell mRNAs are transcribed in the nucleus and exported to the cytoplasm for translation, whereas most RNA viruses replicate in the cytoplasm and their RNAs are transcribed and translated in the cytoplasm. The viral RNA translation is entirely dependent on the host cell translation machinery and thus competes with host mRNA translation. Thus RNA viruses have evolved to ensure efficient translation for viral proteins, and in some cases may stifle the innate immune defense of host. The PRRSV genome is a positive-sense RNA with the 5'-cap and the 3'-polyadenylated tail and its translation is cap-dependent. In the present study, we show that PRRSV blocked the export of host cell mRNAs from the nucleus to the cytoplasm and promoted viral mRNA translation in the cytoplasm. The inhibition of cellular mRNA nuclear export was specific for PRRSV. The PRRSV nsp1-beta protein was specifically localized in the nucleus in cells and played a pivotal role for host cell mRNA nuclear retention. The inhibition of cellular mRNA nuclear export resulted in the enhanced translation of viral mRNA in the cytoplasm. Through the bioinformatics analysis, a motif for SAP (SAF-A/B, Acinus, and PIAS) was identified in nsp1-beta with the consensus sequence of 126-LQxxLxxxGL-135. Site-specific mutagenesis was conducted to substitute residues in the SAP motif to alanine, and a total of seven SAP mutants were constructed. These mutants were examined for their subcellular localization, cellular mRNA nuclear export, and suppressive activities on the host protein synthesis. Exclusive cytoplasmic staining was observed for L126A, R129A, L130A, and L135A, and these mutants did not block the nuclear export of host cellular mRNAs and thus did not suppress the host protein synthesis. PRRSV nsp1-beta was previously shown to inhibit the type I interferon response, and when the SAP mutants were examined for IFN

suppression, the mutants L126A, R128A, R129A, L130A, and L135A were unable to suppress the IFN production, IFN signaling, and TNF-α production. Using the PRRSV reverse genetics, SAP mutant PRRS viruses were generated. Infectious mutant viruses were recovered for PRRSV-K124A, PRRSV-L126A, PRRSV-G134A, and PRRSV-L135A, whereas PRRSV-R128A, PRRSV-R129A, and PRRSV-L130A were non-viable. Among viable mutants, PRRSV-L126A and PRRSV-L135A did not suppress the IFN production. Our study demonstrates that nsp1-beta functions to block the cellular mRNA nuclear export and as a consequence, suppresses the host-protein synthesis. The SAP motif is essential for the nsp1-beta nuclear localization and the cellular mRNA nuclear retention, resulting in the subversion of host protein synthesis and innate immune response.

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Major Biosecurity Issues to Prevent PRRS in Flourishing Pig Farming Enterprise in Nepal

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Pig farming, traditionally adopted as a way of living among few ethnic groups of people is gaining popularity as a commercial enterprise in recent days in Nepal. Besides the lower cost of pig husbandry practice, the availability of improved breeds, lower gestation period, higher litter size, efficient feed conversion ratio, rapid growth, increasing demand of pork and its products, and emerging market opportunities have allured the rural households towards raising pigs. The population of pigs in Nepal is 11, 90,138 according to a report 2013/14 published by Ministry of Agricultural Development (MOAD), Nepal. The pigs contribute 19,269 metric tons of meat annually, which is 6.46% of the total meat production of the country (MOAD, 2014). In order to assess the major biosecurity issues useful to prevent Porcine Reproductive and Respiratory Syndrome (PRRS) in pig farms at rural areas of Nepal, a study was conducted at Sharadanagar Village Development Committee (VDC), Chitwan, Nepal from July-August, 2015. The study includes the field visits, key informant surveys, focal group discussions and interviews with pig farms and Veterinary professionals. The PRRS virus, a RNA virus belonging to Arteriviridae family causes reproductive disorders in adult and older pigs, and respiratory problems in young piglets, thus resulting 20% loss in an annual epidemic. The proper fencing of farms, restriction on visitors, provision of foot and vehicle dipping and medicinal sprays, and changing of cloths by handlers and caretakers need to be followed as the most primary preventive measure. Similarly, the veterinarians and related service givers should also be disinfected properly with utmost precautions before visiting every individual farm. Besides these, it requires serological screening of new pigs (at time of introduction) and existing pigs (periodic), and timely vaccination of the herd. The dissemination of knowledge and skills regarding application and importance of minimum biosecurity measures is crucial to prevent diseases like PRRS, promote the enterprise and establish it as a sustainable means of addressing rural household economy and food security in Nepal.

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Outbreak Investigation Program – A Systematic Approach to PRRS Outbreak Investigations

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Objective: An estimated 4.7–6.4 million weaned pigs were lost in the United States during September 2013–August 2014 due to PEDv. Even in its naïve year, PEDv caused fewer losses than PRRSv causes annually. With outbreaks occurring in 20–40% of breeding herds annually, the high frequency of PRRS outbreaks costs the U.S. swine industry more than $664 million in productivity each year. The industry has yet to learn how to control the introduction of new virus isolates into herds, complicating management or elimination of PRRSv. The objective of this project was to establish an outbreak investigation program to enhance the knowledge of PRRSv spread and

prevention by investigating outbreaks in a timely, efficient, and uniform manner. **Methods**: Breeding herds in Iowa were eligible for inclusion. After being alerted to a PRRS outbreak, the outbreak investigation coordinator gathered historical data collected primarily through the Production Animal Disease Risk Assessment Program (PADRAP) and pre-populated an outbreak investigation form. Veterinary outbreak facilitators, the field epidemiologists, deployed to sites to investigate with herd veterinarians and farm personnel and determined the events most likely to have introduced PRRSv. Outbreak investigation staff completed a written report to share with stakeholders and compiled information into a database. Funding was provided by IPPA.
Results: Breeding herds from area regional control projects (n=23) and herds not associated with regional control projects (n=6) were enrolled in the outbreak investigation project. During January 2015–August 2015, we investigated eight PRRS outbreaks on breed-to-wean farms in Iowa. The events determined to have the highest risk among the eight outbreaks were movement of cull sows (5), employee or repair personnel movement (5), and feed delivery (4). Vehicles used to haul cull sows had been used to haul replacement gilts, rarely practiced lines of separation, and were used with unknown disinfection procedures. Part-time sow farm employees worked at other sites in different stages of production and did not always practice downtime away from other swine. Feed mill equipment was shared with compost equipment in three outbreaks, and feed mill biosecurity practices were largely variable.
Conclusion: Deploying outbreak investigators reduces the time commitment by the herd veterinarians and results in timely identification of gaps in biosecurity. Outbreak investigations provide immediate feedback after an outbreak, helping producers determine the most cost effective biosecurity measures to implement or change. These investigations also emphasize the need for improved communication regarding biosecurity importance and direction, as routes of transmission of PRRSv are not always understood by producers. Increased awareness led producers to implement new biosecurity protocols and enforce compliance with other established protocols. This outbreak investigation program can be adapted and used for other emerging or transboundary diseases, and has recently been used to investigate the first two Seneca Valley virus cases affecting commercial herds in the United States during 2015.

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An evaluation of porcine epidemic diarrhea virus survival in individual feed ingredients in the presence or absence of a liquid antimicrobial

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Introduction: Contaminated complete feed and porcine plasma are risk factors for PEDV introduction to farms and a liquid antimicrobial has been proven useful for reducing risk. This study provides information on the survivability of PEDV across common swine feed ingredients in the presence or absence of the liquid antimicrobial.

Methods: Eighteen ingredients commonly included in commercial swine diets were selected, including 3 grain sources (corn, soybean meal (SBM), dried distillers grains with solubles (DDGS)), 5 porcine by-products (spray-dried plasma, purified plasma, intestinal mucosa, meat and bone meal and red blood cells (RBCs)), 3 vitamin/trace mineral (VTM) mixes (sow, nursery, finishing), 2 fat sources (choice white grease and soy oil), 3 synthetic amino acids (lysine HCL, D/L methionine, threonine), as well as limestone and dry choline chloride. Complete feed and stock PEDV served as controls. Thirty grams of each ingredient were inoculated with 2mL PEDV. A matched set of samples were treated with the formaldehyde-based liquid antimicrobial SalCURB® (LA). All samples (n = 320) were stored outdoors under winter time ambient conditions for 30 days. Samples were submitted on 1, 7, 14 and 30 days post-inoculation (DPI) and tested by PCR and virus isolation (VI). All VI-negative samples were tested by swine bioassay.

Results: Viable PEDV was detected by VI or swine bioassay at 1, 7, 14 and 30 DPI from SBM, DDGS, meat & bone meal, RBCs, lysine HCL, D/L methionine, choice white grease, choline chloride, complete feed and stock virus control and at 7 DPI in limestone and at 14 DPI in threonine. Supplementary testing of complete feed and SBM indicated viable virus out to 45 and 180 DPI, respectively. All other samples were negative by VI and bioassay. In contrast, treatment with LA inactivated PEDV across all ingredients on 1 DPI and induced RNA reduction over time.

Conclusions: Under the conditions of this study, PEDV viability in feed was influenced by ingredient with extended survival in SBM. Furthermore, LA treatment rendered virus inactive, independent of ingredient type. This study provides the initial proof of concept demonstrating extended survival of PEDV in feed ingredients. This new information improves our understanding of the risk of PEDV infection via feed at the domestic level and provides justification for further studies investigating transboundary risk.

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Network analysis applied to Classical Swine Fever epidemiology in Cuba

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Classical swine fever (CSF), is one of the most important viral disease of swine. In Cuba, CSF is an endemic disease, causing significant economic losses annually in the swine industry. The Cuban Veterinary Authority with the collaboration of research centers and pigs producers, arranged a strategy of eradication by zones, in agreement with CSF Eradication Plan for America projected by Food and Agriculture Organization (FAO). Contagious diseases can spread between holdings and one of the most important ways is the movement of live animals. Network analysis has previously been used to provide summaries of animal movements, and to improve the understanding of interconnectivity among farms. Those methods can help to explore the potential of speed and range of spread of an infectious agent.

In Cuba, the qualitative studies of animal movements as part of livestock production has been mainly applied to explain the past or ongoing epidemics, but the statistical assessment has not been applied to the disease spread analysis and management of risk. The network analysis is an important tool for a better understanding of the structure, links and animal flow, and to identify nodes with higher or lower risk of introduction and/or dissemination of diseases through the commercial relationships, which help to developing strategies for risk based surveillance. The aim of our study was to identify higher-risk districts for CSF outbreak occurrences and those districts with high potential to spread the disease on the pig movement network. The study can make a decisive contribution to the surveillance activities, implementation of control measures and hence to disease eradication. Pinar del Río has received pigs from three municipalities of the neighbor province. Those represent the 2.3% (26 movements) of the analyzed movements and were introduced 4431 animals by this way. It just occurs from July 07, 2010 to June 2011, because according to the implemented policies to eradicate the disease in Pinar del Río the introduction of live animals from other provinces has been forbidden since 2011. Our network has 1121 animal movements and 212 links among districts. In the studied period were moved 127 653 animals, an average of 41 (±11) shipments and 4 255 animals per month. The most animals moved in the province are intended to fattening. Only the ICC showed a significant association with the occurrence of CSF outbreaks ($p = 0.045$). Districts with ICC between 8 and 11 have higher risk (OR = 5.1, 95% CI: 1.04 – 25.04, $p=0.036$) than nodes with ICC (baseline category), and nodes with ICC between 1 and 7 showed no significant difference with the reference category. The network analysis to identify districts with more probability of outbreak occurrence and where is more likely to disease spreading , brought useful results for understanding the CSF dynamic , and getting support to policy makers, producers, veterinary authority and researchers. It contributes to improve the decision-making process for the CSF control an eradication strategy. Future studies will include community detection, other variables and a link to geostatistics results, improving the approach.

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Evaluating optimal strategies for regional control of porcine reproductive and respiratory syndrome (PRRS): a collective choice problem approach

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Purpose: Swine producers face incentives to control disease depending on the type and expected severity of the disease, the feasibility and cost of containment and the expected economic impact on farm profits. Furthermore, system structure, knowledge about other producers, and government policy are likely to influence disease control. Because of there is no an official program for containment of porcine reproductive and respiratory syndrome (PRRS), a high-impact endemic disease, some states have implemented voluntary regional control projects (RCPs). This study aims to explore and quantify individual and aggregated effects of strategies taken by a set of producers from a RCP on PRRS control.
Methods: This research is based on a system dynamics simulation approach, which assumes that disease spread between farms is related to farmers' individual and collective choice of production strategies, is used. We assume that disease risk is an endogenous factor that depends on factors such as health, production investments, and management decisions at production sites (e.g., vaccination, all in/all out, and unit size), system elements linking production sites (e.g., feed suppliers and animal transport modes), and the nature of firm agreements (e.g., sales contract incentives, system-based management).
Results: The model formulation here allows to set several putative scenarios on individual and/or collective selection of strategies taken by producers, with several disease outcomes. The resulting disease model allows linking it with a system dynamics economic model to compare and contrast different strategies to identify the economic optimal (or optimums) on the control of PRRS, as well as estimate of impacts generated due to the selection, or rejection, of strategies assessed.
Conclusions: A theoretical model to estimate optimal economic strategies for swine producers was created. Scenarios show that disease dynamics is tied to production preferences. To generate effective PRRS control actions, it is important to consider regional setting and interactions between producers and other suppliers. The proposed study will later utilize data from RCPs such as the Minnesota Voluntary Regional PRRS Elimination Project (RCP-N212) to test alternative approaches to PRRS regional control. This will provide a baseline for the assessment of state and federal control programs.

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Measuring progress on the control of porcine reproductive and respiratory syndrome (PRRS) at a regional level: the Minnesota N212 regional control project (RCP) as a working example

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Purpose: Due to the highly transmissible nature of porcine reproductive and respiratory syndrome (PRRS), implementation of regional programs to control the disease is critical. Because PRRS is not reported in the US, numerous voluntary regional control projects (RCPs) have been established. However, the effect of RCPs on PRRS control has not been assessed. This study aims to quantify the extent to which RCPs contribute to PRRS control by proposing a novel methodological framework to evaluate the progress of RCPs. We anticipate the establishment of a benchmark that may facilitate comparisons among RCPs in the US.
Methods: Information collected between July 2012 and July 2015 from the RCP-N212 located in MN was used. Farm enrollment and demographics (e.g. composition of farms with sows = SS and without sows = NSS) were evaluated. By using general linear mixed-effects models, active participation of farms enrolled in the

RCP-N212, defined as the decision to share (or not to share) PRRS status, was evaluated and used as a predictor, along with other variables, to assess the PRRS trend over a 37-month period. Additionally, spatial and temporal patterns of the disease were investigated.

Results: The number of farms enrolled in RCP-N212 and its geographical coverage has increased over time, but the proportion of SS and NSS, representing 23% and 77% of farms, respectively, did not vary significantly over time. A significant increasing ($p<0.001$) trend in farmers' decision to share PRRS status, but with NSS producers less willing to report and a larger variability between than within counties, was observed. The incidence of PRRS significantly ($p<0.001$) decreased over the study period, showing a negative correlation between degree of participation and occurrence of PRRS. In turn, farm density at the county level was positively related with incidence of PRRS ($p=0.02$), while the type of farm and proportion of stable farms in counties were not related with the occurrence of PRRS ($p>0.05$). Despite a noted decrease in PRRS, significant spatio-temporal patterns of incidence of the disease over 3-weeks and 3-kms during the entire study period were identified.

Discussion: This study established a systematic approach to quantify the effect of RCPs on PRRS control. Despite an increase in number of farms enrolled in the RCP-N212, active participation is not ensured. By evaluating the effect of participation on the occurrence of PRRS, the value of sharing information among producers may be demonstrated, in turn justifying the existence of RCPs. However, incentives and deterrence of participation in RCPs as well as other collaborative strategies to control PRRS at a regional level must be subject to further studies.

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