PROCEEDINGS OF THE
XVIIITH INTERNATIONAL SYMPOSIUM
OF THE WORLD ASSOCIATION OF VETERINARY
LABORATORY DIAGNOSTICIANS (WAVLD)

WAVLD 17
Saskatoon, Canada
June 15-18, 2015

PRESENTED BY THE CANADIAN ANIMAL HEALTH LABORATORIANS NETWORK (CAHLN)
Canadian Animal Health Laboratorians Network (CAHLN) | Prairie Diagnostic Services Inc.

17th International Symposium of the World Association of Veterinary Laboratory Diagnosticians (WAVLD)

11th OIE Seminar

June 15-18 2015
Saskatoon, Canada

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Welcome To Canada!

It is with great pleasure that we welcome delegates for the 17th World Association of Veterinary Laboratory Diagnosticians (WAVLD) Symposium to Canada.

Our goal for this conference has been to deliver a truly memorable event for delegates of the WAVLD Symposium in Saskatoon. In keeping with the WAVLD mandate we have committed to facilitating the exchange of information from experts throughout the world and to create opportunities for professional development and networking for laboratorians, industry, academia and government.

We thank the many delegates from around the world that have submitted abstracts and posters to share with their colleagues as part of the scientific program. Their contributions are a critical and important part of the program. We also thank our OIE partners. Their involvement in this program is fundamental in providing context to what is happening internationally within animal health and to ensure that we are all working together through partnerships, science, and regulation to most effectively manage and control animal disease.

We have been amazed and thankful for the support from our suppliers, as sponsors and through their participation in the industry exhibition. A number have also contributed abstract presentations or posters to the program. This is strong evidence of the partnership that is critical and necessary for us, as laboratorians, to deliver services to our client groups and to ensure we are able to play our part in supporting and furthering our broader mandates and contributions to One Health.

While our laboratory at Prairie Diagnostic Services has been the lead on this conference, this has truly been a cross Canada initiative through our national diagnostic organization, the Canadian Animal Health Laboratorians Network (CAHLN). I would like to thank members of our organizing committee, our scientific committee, and our own staff at Prairie Diagnostic Services for their contribution of time and energy to developing and delivering this program. I would also like to thank Saskatoon Tourism and the City of Saskatoon for their considerable support in bringing the program to Saskatoon and in supporting delivery of the conference. Last but certainly not least, is our thanks to Dr. Craig Carter, the Executive Director and champion of WAVLD who provided unending enthusiasm and support to our team throughout the last 2 years.

We welcome the friendships and future partnerships that will be forged by this conference and encourage all of our delegates to make the most out of the networking opportunities that are available to enhance and build new partnerships between individuals, laboratories and with industry.

We welcome the world to Saskatoon. Enjoy!

Best regards,

Marilyn Jonas
Chair, WAVLD 2015
President Elect, CAHLN
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17th International Symposium of the World Association of Veterinary Laboratory Diagnosticians

WAVLD 2015
Keynote Speakers
NEW TECHNOLOGIES

Andrew Soldan  
*Animal and Plant Health Agency*  
Addelstone, United Kingdom

Andrew worked as a vet in mixed general practice in Yorkshire, and then following an MSc in Tropical Veterinary Medicine, worked in Malawi. During this time he specialised in laboratory diagnosis of disease and control methods for tick borne disease in local cattle. For the latter work he was awarded a Doctorate of Veterinary Medicine. On return from Africa he worked as a government pathologist before another spell in mixed practice. In 1999 he started working for the Veterinary Laboratories Agency (VLA) and was heavily involved in the laboratory response to the CSF outbreak in 2000 and the FMD outbreak in 2001. He has had various roles within VLA and its successors, including Head of Testing, Veterinary Director, Commercial Director, redesigning the veterinary scanning surveillance system and leading a merger to create the Animal and Plant Health Agency. Andrew has been involved in the development and validation of new tests, bringing them into routine use in an ISO17025 accredited laboratory. He has taken a special interest in tests that can be used outside a specialist laboratory informed by his time working in Africa, in general veterinary practice and in disease outbreaks. Andrew was a founding member of the European Association of Veterinary Laboratory Diagnosticians and its first President.

Bruce Akey  
*BS MS DVM*  
*Texas A&M Veterinary Medical Diagnostic Laboratory*  
Texas, United States

Having been engaged in veterinary diagnostic laboratory science for over 30 years, Dr. Akey currently serves as the Director of the Texas A&M Veterinary Medical Diagnostic Laboratory which is accredited by the American Association of Veterinary Laboratory Diagnosticians (AAVLD) and ISO 17025, and a founding member of the US National Animal Health Laboratory Network (NAHLN). He also served as Assistant Dean for Diagnostic Operations and Executive Director of the Animal Health Diagnostic Center at Cornell University, and before that as Chief of the Bureau of Laboratory Services for the Virginia Department of Agriculture and Consumer Services. Dr. Akey also has extensive experience in regulatory veterinary medicine, having served as an Assistant State Veterinarian in both New York and Virginia.

Dr. Akey was President of the AAVLD in 2000, was presented the E. P. Pope Memorial Award in 2004, voted a Life Member in 2007 and is still active in the Association. At the federal level, he serves as a Co-Chair of the NAHLN Methods Technical Working Group, member of the NAHLN Coordinating Council and NAHLN Information Technology Committee as well as the National Animal Health Information Technology Board of the USDA.

Dr. Akey earned a BSc (Biology) from the College of William and Mary in Virginia, an MSc (Parasitology) from the University of Florida and the DVM degree from the University of Minnesota.
Soren Alexandersen

DVM, PhD, DVSc, FRCPath, MRCVS
National Centres for Animal Disease,
Canadian Food Inspection Agency
Winnipeg, Canada

Soren Alexandersen is a Veterinary Pathologist, Virologist and Epidemiologist specialising in molecular pathogenesis, epidemiology and control of virus infections since 1982. He has worked at the NIH in Montana and at Iowa State University in the USA and has previously been Assistant and Associate Professor of Veterinary Pathology, Research Professor of Molecular Pathobiology and Professor and Chair of Veterinary Virology at the Veterinary and Agricultural University in Denmark. Professor Alexandersen was then Director of the Danish Veterinary Institute for Virus Research at the Island of Lindholm for 5 years before leaving for the Institute for Animal Health in Pirbright, England in 1999. At Pirbright he was Head of Experimental Epidemiology focussing on foot-and-mouth disease and swine vesicular disease. In August 2004 he returned to the Danish Veterinary Institute for Virus Research, Denmark, as Research Professor and Head of Section to work on serious OIE listed virus infections of livestock. In 2008 Professor Alexandersen became the Director of the Canadian Food Inspection Agency's National Centre for Foreign Animal Disease (NCFAD) in Winnipeg, Canada and from 2010 to 2015 served as the Executive Director of the National Centres for Animal Disease (NCAD) including the NCFAD laboratory in Winnipeg and the Lethbridge Laboratory in Alberta. He has also been Adjunct Professor of Pathology and Exotic Virology at the Faculty of Life Sciences of the University of Copenhagen. He has worked and given invited lectures in most parts of the world and has published more than 130 international scientific papers. Professor Alexandersen holds a Doctor of Veterinary Medicine, a PhD in Veterinary Pathology and a DVSc in Molecular Virology from the Veterinary and Agricultural University in Denmark, and is a Fellow of the Royal Society of Pathologists and a Member of the Royal College of Veterinary Surgeons in the United Kingdom.

Daral Jackwood

The Ohio State University,
Ohio, United States

Dr. Daral J. Jackwood is a Professor in the College of Veterinary Medicine, Food Animal Health Research Program at The Ohio State University, Ohio Agricultural Research and Development Center. He earned his B.S. from the University of Delaware and his M.S. and Ph.D. at The Ohio State University.

Dr. D. Jackwood is a Molecular Virologist and his primary area of research is the study of immunosuppressive viruses particularly the Birnavirus known as infectious bursal disease virus (IBDV). His studies have examined the evolution of IBDV strains around the world. This work has focused on the molecular characteristics of IBDV strains; including examining the molecular basis for immunogenic, antigenic and pathogenic properties of these viruses. Serology and molecular techniques are being used to differentiate the various antigenic and pathogenic types and Dr. Jackwood is examining the potential of multivalent vaccines for the control of infectious bursal disease. He has over 80 peer reviewed articles published in scientific journals and more than 175 abstracts and proceedings published from presentations given at scientific conferences. His work is internationally recognized and he has given invited talks on IBDV in numerous countries around the world.
DETECTION, MANAGEMENT AND CONTROL OF NEW AND EMERGING DISEASE WILDLIFE AND ONE HEALTH

Dick Hesse
*Kansas State Veterinary Diagnostic Laboratory,*
Kansas State University,
Kansas, United States

Dick Hesse is the Director of diagnostic virology at the Kansas State's Veterinary Diagnostic Laboratory in the College of Veterinary Medicine, and is a professor of diagnostic medicine and pathobiology.

Hesse received his master's from South Dakota State University, with a focus on bovine respiratory viruses, and his doctorate from the University of Nebraska, with a focus on porcine rotavirus. His many honors include: the Army Distinguished Service Medal for Lassa Fever research; membership in the Gamma Sigma Delta honor society; the Phi Zeta Honor Society of Veterinary Medicine; the Schering Plough Excellence Award for development of a porcine respiratory and reproductive syndrome vaccine; and the Kansas Veterinary Medical Association Distinguished Service Award. The bulk of his career has involved research and development of animal vaccines in the private sector. He has authored or co-authored more than 70 publications, presentations and/or patents. In addition, he has led the development of at least 12 U.S. Department of Agriculture-licensed vaccines.

Hesse’s most recent focus has been on the pathogenesis, improved diagnostic assays and vaccine development for the recently emerged swine enteric viruses; porcine epidemic diarrhea virus (PEDV) and porcine deltacorona virus (PDCoV); and continuing work on porcine respiratory and reproductive syndrome, (PRRS), and porcine circovirus-associated disease (PCVAD). He is also actively engaged in developing rapid diagnostic assays and vaccines for zoonotic, foreign animal and emerging/re-emerging diseases.

Thijs Kuiken
*Erasmus University Medical Centre,*
Rotterdam, The Netherlands

After graduating as a veterinarian from Utrecht University in 1988, Dr Kuiken worked in London, England, for three years as a marine mammals stranding coordinator for England and Wales. There he discovered that a large proportion of small cetacean strandings on the British coast were caused by accidental entrapment in fishing nets. He did his Ph.D. from 1993 to 1998 at the University of Saskatchewan, Saskatoon, Canada, where he characterized the epidemiology of Newcastle disease in the double-crested cormorant. Following his Ph.D., Dr Kuiken specialized in pathology and moved to the Department of Viroscience at the Erasmus University Medical Centre in Rotterdam, The Netherlands, in 1999, where he is now Professor of Comparative Pathology. He was part of the team that identified the etiological agent of Severe Acute Respiratory Syndrome (SARS), and determined that avian H5N1 influenza virus was highly virulent for cats and other carnivores. Recent achievements of his group include the discovery of novel routes of entry of influenza virus in mammalian hosts, identification of an unusual pattern of attachment of the recently emerged H7N9 influenza virus to the human respiratory tract, and elucidation of the clinical effect of influenza in the wild bird reservoir. His current focus is the pathogenesis of influenza-associated pneumonia and encephalitis, comparison of viral infections between bats and people, and identification of underlying factors for viruses to cross the species barrier from wildlife reservoirs to humans.
Thierry Work is a wildlife disease specialist and project leader of the Honolulu Field Station. He has a BS degree in entomology from Texas A&M University, a masters of science in entomology from University of California, Davis (UCD), a doctor of veterinary medicine and a masters of preventive veterinary medicine from UCD. He completed a residency in wildlife medicine at UCD and worked for California Department of Fish and Game as a wildlife veterinarian before coming to Hawaii in 1992. Interests include developing ways to use health monitoring of wildlife to help better manage natural resources and recover endangered species in terrestrial and marine ecosystems.

Gary Wobeser
Professor Emeritus, Department of Veterinary Pathology, Western College of Veterinary Medicine, University of Saskatchewan
Saskatoon, Canada

Raised on a mixed farm in southern Saskatchewan.

Education: BSA (Fisheries and Wildlife Management), U. of Toronto; MSc (Zoology) U. of Guelph; DVM, U. of Guelph; PhD (Veterinary Pathology) U. of Saskatchewan.

Joined the Department of Veterinary Pathology, University of Saskatchewan in 1974, and remained until retirement in 2010. Founding co-director of the Canadian Cooperative Wildlife Health Centre.

TURNING LABORATORY DATA INTO INTELLIGENCE

Lina Mur
Institute for Infectious Animal Diseases,
Texas, United States

Lina Mur, DVM, Ph.D. is a research scientist in the Institute for Infectious Animal Diseases (IIAD), a Department of Homeland Security Science and Technology Center of Excellence.

Dr. Mur earned her PhD on the University Complutense of Madrid Spain, OIE Reference laboratory for African swine fever (ASF). During these years, she focused on the research of new strategies for preventing and controlling ASF in different affected scenarios (Africa, Sardinia and Russian Federation). Her expertise and research interests include risk assessment studies, surveillance and control strategies for transboundary animal diseases. She has also worked on the development and evaluation of diagnostic tests for alternative samples (i.e. oral fluids).

Currently at IIAD she is focused on providing epidemiological expertise related to data analysis and development of novel methods for temporal and spatial analysis of syndromic surveillance and laboratory data, as well as production, movement, and other animal health information collected through IIAD’s AgConnectTM suite of tools.

Crawford Revie
Atlantic Veterinary College, University of Prince Edward Island
Charlottetown, Canada

Dr. Crawford Revie is the Canada Research Chair in Epi-Informatics, which he defines as, “exploring new ways to use techniques from informatics - such as data mining, data-driven modelling and semantic web technologies - to gain a better understanding of disease epidemiology”. The main focus of his research considers novel methods to extract and organize knowledge that exists in large/complex epidemiological data sets. A number of doctoral and post-doctoral researchers in his group have focused on using techniques from statistics and machine learning to extract and exploit surveillance intelligence from data that exists in veterinary laboratory systems.

Crawford has degrees in Computing Science, Microprocessor Design and Industrialisation. His doctoral work in Statistics and Mathematical Modelling involved the creation of models describing host-parasite interactions in aquaculture. Most of his applied research has been carried out with colleagues from veterinary or human medicine, and he enjoys the challenge as well as opportunities that come from working at the ‘bleeding edge’ of inter-disciplinary research. Prior to moving to Canada in 2008, Crawford was based in Scotland and continues to collaborate on research with colleagues in the UK as well as leading a number of on-going projects in East Africa.
# WAVLD 2015 Program Schedule

<table>
<thead>
<tr>
<th>Registration - Lobby</th>
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<tr>
<th>Exhibitor Hours - Grand Salon</th>
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<tbody>
<tr>
<td>Tuesday &amp; Wednesday - 9:00 am - 4:00 pm, Thursday 9:00 am - 11:30 am</td>
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<tr>
<th>Speaker Ready Room - Gallery Suite I</th>
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<tbody>
<tr>
<td>Tuesday &amp; Wednesday - 7:30 am - 4:00 pm, Thursday 7:30 am - 10:00 am</td>
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<tr>
<th>Monday, June 15, 2015</th>
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<th>Wednesday, June 17, 2015</th>
<th>Thursday, June 18, 2015</th>
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<tr>
<td><strong>OFF SITE TOUR</strong> - Meet at TCU Place Lobby</td>
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<td><strong>PLENARY III Detection, Management and Control of New and Emerging Disease Sponsored by VMRD Centennial Hall AB</strong></td>
<td><strong>PLENARY V Turning Laboratory Data into Intelligence Sponsored by Saskatchewan Ministry of Agriculture Centennial Hall AB</strong></td>
</tr>
<tr>
<td>Agriculture in Saskatchewan Tour Sponsored by Advanced Technology 9:00 am to 5:00 pm</td>
<td><strong>PLENARY I New Technologies Centennial Hall AB</strong></td>
<td><strong>PLENARY II Control and Management of Endemic Disease Sponsored by IDEXX Centennial Hall AB</strong></td>
<td><strong>PLNARY VI</strong></td>
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<td><strong>PLENARY II</strong></td>
<td><strong>COFFEE &amp; EXHIBITORS &amp; POSTERS Grand Salon</strong></td>
<td><strong>PLENARY II Control and Management of Endemic Disease Sponsored by IDEXX Centennial Hall AB</strong></td>
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<td><strong>PLENARY III Detection, Management and Control of New and Emerging Disease Sponsored by VMRD Centennial Hall AB</strong></td>
<td><strong>SESSION 1 Centennial Hall AB</strong></td>
<td><strong>SESSION 2 Gallery AB</strong></td>
<td><strong>OIE SEMINAR Gallery CD 10:15 am - 12:00 pm</strong></td>
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<tr>
<td><strong>SESSION 1 Centennial Hall AB</strong></td>
<td><strong>SESSION 2 Gallery AB</strong></td>
<td><strong>SESSION 3 Centennial Hall AB</strong></td>
<td><strong>SESSION 7 Sponsored by Zoetis Gallery AB</strong></td>
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<td><strong>SESSION 2 Gallery AB</strong></td>
<td><strong>SESSION 3 Centennial Hall AB</strong></td>
<td><strong>SESSION 3 Centennial Hall AB</strong></td>
<td><strong>SESSION 8 Gallery CD</strong></td>
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<td><strong>SESSION 3 Centennial Hall AB</strong></td>
<td><strong>SESSION 4 Sponsored by Biovet Gallery AB</strong></td>
<td><strong>SESSION 4 Sponsored by Biovet Gallery AB</strong></td>
<td><strong>ITLALIAN LUNCH Centennial Hall AB Sponsored by Thermo Fisher Scientific, Inc. SYMPOSUM CLOSE</strong></td>
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<td><strong>SESSION 4 Sponsored by Biovet Gallery AB</strong></td>
<td><strong>SESSION 5 Sponsored by Tetracore Centennial Hall AB</strong></td>
<td><strong>SESSION 5 Sponsored by Tetracore Centennial Hall AB</strong></td>
<td><strong>TASTE OF SASKATCHEWAN BANQUET, PRESENTATIONS, ENTERTAINMENT Sponsored by the City of Saskatoon Centennial Hall AB</strong></td>
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<tr>
<td><strong>SESSION 5 Sponsored by Tetracore Centennial Hall AB</strong></td>
<td><strong>SESSION 6 Gallery AB</strong></td>
<td><strong>SESSION 6 Gallery AB</strong></td>
<td><strong>OFF SITE TOUR - Meet at TCU Place Lobby Saskatoon's Meewasin River Valley 1:30 pm to 4:30 pm</strong></td>
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<td><strong>SESSION 6 Gallery AB</strong></td>
<td><strong>SESSION 7 Sponsored by Zoetis Gallery AB</strong></td>
<td><strong>SESSION 7 Sponsored by Zoetis Gallery AB</strong></td>
<td><strong>WELCOME RECEPTION Sponsored by Western College of Veterinary Medicine Grand Salon 6:30 pm - 9:00 pm</strong></td>
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<td><strong>SESSION 7 Sponsored by Zoetis Gallery AB</strong></td>
<td><strong>SESSION 8 Gallery CD</strong></td>
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<td><strong>WILD WILD WEST INFORMAL EVENING Sponsored by QIAGEN Western Development Museum 6:00 pm - 9:00 pm</strong></td>
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<td><strong>SESSION 8 Gallery CD</strong></td>
<td><strong>WELCOME RECEPTION Sponsored by Western College of Veterinary Medicine Grand Salon 6:30 pm - 9:00 pm</strong></td>
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- **CAVP Forensic Pathology Workshop and AGM**
  - Room 134, College of Arts and Sciences, University of Saskatchewan
  - 8:00 am - 5:00 pm

- **TSE (Canadian Laboratories) Meeting**
  - Room 298, College of Arts and Sciences, University of Saskatchewan
  - 8:00 am - 5:00 pm

- **Canadian Animal Health Laboratory Network (CAHLN) Meeting**
  - Gallery CD
  - 8:00 am - 9:30 am

- **PLENARY I New Technologies**
  - Centennial Hall AB
  - 8:00 am - 9:45 am

- **PLENARY II Control and Management of Endemic Disease**
  - Centennial Hall AB
  - 1:30 pm - 3:15 pm

- **PLENARY III Detection, Management and Control of New and Emerging Disease**
  - Centennial Hall AB
  - 1:30 pm - 3:15 pm

- **PLENARY IV Wild Life and One Health**
  - Centennial Hall AB
  - 1:30 pm - 3:15 pm

- **PLENARY V Turning Laboratory Data into Intelligence**
  - Centennial Hall AB
  - 8:00 am - 9:45 am

- **COFFEE & EXHIBITORS & POSTERS**
  - Grand Salon
  - 10:00 am - 10:45 am

- **PLENARY VI Turning Laboratory Data into Intelligence**
  - Centennial Hall AB
  - 8:00 am - 9:45 am

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  - Centennial Hall AB
  - 8:00 am - 9:45 am
Monday, June 15, 2015  All events at TCU Place, 35 - 22nd Street East, Saskatoon or otherwise stated.

12:00 pm - 8:00 pm  REGISTRATION
Lobby

8:00 am - 5:00 pm  Canadian Association of Veterinary Pathologists Forensic Pathology Workshop and Annual General Meeting
*Off site at Room 134, College of Arts and Sciences, University of Saskatchewan*

9:00 am - 5:00 pm  OFF SITE TOUR - Meet at TCU Place Lobby
There is limited space available for each tour. Pre-registration is required.

**Agriculture in Saskatchewan Tour**
_Sponsored by Advanced Technology Corp_
Take a “road trip” outside of Saskatoon to learn about the broad range of agriculture production in Saskatchewan. Features a stop at a working ranch. ($75.00, includes lunch)

12:00 pm - 1:30 pm  TSE (Canadian Laboratories) Meeting
*Off site at Room 298, College of Arts and Sciences, University of Saskatchewan*

1:00 pm - 5:00 pm  OFF SITE TOUR - Meet at TCU Place Lobby
There is limited space available for each tour. Pre-registration is required.

**Saskatoon Science Tour**
_Sponsored by Gyver Industries_
Showcases the University of Saskatchewan with specific stops at the diagnostic lab, the vet college, and two internationally recognized research institutions. $25.00

6:30 pm - 9:00 pm  WELCOME RECEPTION
Grand Salon
_Sponsored by the Western College of Veterinary Medicine_

Join us at the opening reception where you can meet and greet your colleagues and warm up for the sessions the following day. Hosted at TCU Place on Monday, the event will feature an amazing selection of complimentary appetizers, refreshments. As part of the program, Dr. Martine Dubuc, Vice-President of Science Branch for the Canadian Food Inspection Agency, Chief Food Safety Officer for Canada, and the OIE Delegate for Canada will provide a rare glimpse of her career and “life” path that has led to her work today. The reception is included with registration. (presentation at 7:15 pm)
**Tuesday, June 16, 2015**  All events at TCU Place, 35 - 22nd Street East, Saskatoon or otherwise stated.

<table>
<thead>
<tr>
<th>Time</th>
<th>Event</th>
<th>Location</th>
<th>Details</th>
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<tbody>
<tr>
<td>7:15 am - 4:00 pm</td>
<td>REGISTRATION</td>
<td>Lobby</td>
<td>Diagnostics in resource-poor situations: a reliable test result even when it is raining and dark</td>
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<td><strong>Andrew Soldan</strong>, Animal and Plant Health Agency, United Kingdom</td>
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<td><strong>Future vision</strong></td>
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<td><strong>Bruce Akey</strong>, Texas A&amp;M Veterinary Medical Diagnostic Laboratory, USA</td>
</tr>
<tr>
<td>8:00 am - 10:00 am</td>
<td>WELCOME AND OPENING REMARKS</td>
<td>Centennial Hall AB</td>
<td><strong>PLENARY I: New Technologies</strong></td>
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<td><strong>Future vision</strong></td>
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<td></td>
<td><strong>Bruce Akey</strong>, Texas A&amp;M Veterinary Medical Diagnostic Laboratory, USA</td>
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<tr>
<td>10:00 am - 10:45 am</td>
<td>COFFEE &amp; EXHIBITORS</td>
<td></td>
<td>POSTERS - Authors for posters #48-63 to be present to answer questions</td>
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<td>Grand Salon</td>
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<tr>
<td>10:45 am - 12:00 pm</td>
<td>CONCURRENT SESSION 1</td>
<td>Centennial Hall AB</td>
<td><strong>001 Rapid and sensitive detection of Classical swine fever virus on a portable compact user-friendly device with automatic display of results</strong></td>
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<td><strong>Oliver Lung</strong>, John Pasick, Mathew Fisher, Cody Buchanan, Anthony Erickson, Aruna Ambagala</td>
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<td><strong>002 Detection of bovine and equine rotaviruses by reverse transcription-insulated isothermal polymerase chain reaction on a field-deployable device</strong></td>
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<td><strong>Rebecca Wilkes</strong>, Mohamed Soltan, Yun-Long Tsai, Chuan-Fu Tsai, Hsiu-Hui Chang, Pei-Yu Lee, Hsiao-Fen Chang, Hwa-Tang Wang</td>
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<td><strong>003 A novel molecular diagnostic tool for Equine arteritis virus detection and characterization</strong></td>
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<td><strong>Delphine Gaudaire, Nicolas Berthet, Aymeric Hans</strong></td>
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<td><strong>004 Solutions for rapid detection of pathogen nucleic acids in liquid animal samples: introducing QIAGEN’s new cador MagBead Kit</strong></td>
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<td><strong>Christine Gaunitz, Marco Labitzke, Oliver Sasse, Stephen Hennart, Phoebe Loh, Claudia Engemann</strong></td>
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<td><strong>005 Performance evaluation of the Fluidigm BioMark™ platform for high-throughput microbe monitoring in salmon</strong></td>
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<td><strong>Kristi Miller, Raphael Vanderstichel, Angela Schulze, Shaorong Li, Amy Tabata, Ian Gardner</strong></td>
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<td><strong>007 Efficacy of commercial infectious bursal disease (IBD) vaccines against “variant IBD virus-NC171” infection in broiler chickens</strong></td>
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<td><strong>Shanika Kurukulasuriya</strong>, Davor Ojkic, Philip Wilsson, Thushari Gunawardana, Shalini Kurukulasuriya, Betty Lockerbie, Afic Jasna, Susantha Gomis</td>
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<td><strong>008 Oligodeoxynucleotides containing CpG motifs (CpG-ODN) as an immunoprotective agent against viral diseases in broiler chickens</strong></td>
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<td><strong>Thushari Gunawardana, Marianna Foldvari, Shelly Popowich, Suresh K Tikoo, Philip Wilsson, Betty Lockerbie, Shanika Kurukulasuriya, Ashish Gupta, Ruwani Karunarathne</strong></td>
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<td><strong>009 Development and evaluation of a multiplex molecular assay for poultry pathogens with lab-on-chip</strong></td>
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<td><strong>Yook Wah Choi, Zhimer Li, Bharati Kadamb Patel, Mitsuharu Sato, Brian Meehan, Songhua Shi, David Williams, Chris Morrissy, Axel Colling, Waikwan Wong, Taoqi Huangfu</strong></td>
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<td><strong>010 Identification of bacterial species isolated from non-viable chicken embryos in Western Canadian hatcheries</strong></td>
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<td><strong>Ruwani Karunarathna, Morgan Wawryk, Chenfeng Yu, Shelly Popowich, Musangu Ngeleka, Susantha Gomis</strong></td>
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<tr>
<td>12:00 pm - 1:30 pm</td>
<td>LUNCH &amp; EXHIBITORS &amp; POSTERS</td>
<td>Grand Salon</td>
<td>Qiagen Seminar</td>
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<td>Gallery CD</td>
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<td>1:30 pm - 3:15 pm</td>
<td>PLENARY II: Control and Management of Endemic Disease</td>
<td>Centennial Hall AB</td>
<td>Control and management of endemic Disease - an international Dane’s perspective</td>
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<td>Social media: <strong>Soren Alexandersen</strong>, Winnipeg, Canada</td>
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<td>Molecular evolution and the consequential diagnostic challenges for infectious bursal disease viruses</td>
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<td><strong>Daran Jackwood</strong>, The Ohio State University, Ohio, United States</td>
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<tr>
<td>3:15 pm - 3:45 pm</td>
<td>COFFEE &amp; EXHIBITORS</td>
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<td>POSTERS - Authors for posters #64-78 to be present to answer questions</td>
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<td>Grand Salon</td>
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<td>Time</td>
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| 3:45 pm - | CONCURRENT SESSION 3                        | Centennial Hall AB| Culture and PCR detection of *Campylobacter fetus* spp.*venerealis* from preputial scrapings inoculated into transport and In-Pouch TF media, and saline<br><br>Musangu Ngeleka*, Steve Hendrick, Anju Tumber
| 5:15 pm   | Comparison of different sample preparation methods for bovine vaginal mucus for detection of *Campylobacter fetus* subsp. *venerealis* with real-time PCR<br><br>Yaroslav Yarokhno*, Janet Hill, Bonnie Chaban, Steve Hendrick
|           | Intra-uterine bacterial species and *Escherichia coli* virulence genes could allow predicting the risk of developing postpartum metritis in dairy cows<br><br>Flavien Ndongo Kassé*, John Morris Fairbrother, Jocelyn Dubuc
|           | Trends of bacterial antimicrobial resistance associated with cattle affected with bovine respiratory disease complex (BRDC) in Missouri, USA<br><br>William Fales, Thomas Reilly, Irene Ganjam, Jesse Bowman
|           | Field evaluation of an improved *Anaplasma* antibody cELISA using recombinant MSP5-GST and evaluation of cross-reactivity of *Ehrlichia* antibodies<br><br>Chungwon Chung, Carey L. Wilson, Grace Chung, Ethan Adams, Glen Scole, Stephen Lee, Betty Golsteyn-Thomas, Steve Hennager
|           | Defining the long-term duration of parasitemia and antibody response in cattle infected with *Babesia bovis* and evaluating serodiagnostic tools<br><br>Chungwon Chung, Carlos Suarez, Carey L. Wilson, Chandima-Bandara Bandaranayaka-Mudiyanselage, Joanna Rzepka, Amanda L. Grimm, Grace Chung, Stephen S. Lee
|           | CONCURRENT SESSION 4                        | Gallery AB        | Development and validation of complete workflow solution for SIV testing<br><br>Angela Burrell, Rohan Shah, Darcy Myers, Ivan Leyva Baca, Catherine O’Connell
|           | Detection of type 2 PRRS virus in the fetal implantation sites of pregnant gilts<br><br>Predrag Novakovic*, John C.S. Harding, Susan E. Detmer
|           | Assessment of the antibody response in pigs directed against *Porcine circovirus* 2<br><br>Cristina Solis Worsfold*, Narges Nourozieh, Rkia Dardari, Regula Waekerlin, Frank Marshall, Markus Czub
|           | Measuring by quantitative PCR the critical number of *Lawsonia intracellularis* that cause production losses on a commercial pig farm<br><br>Alison Collins
|           | Next-generation sequencing and quantitative PCR can identify the impact of antimicrobials on the abundance of bacteria in pig feces<br><br>Alison Collins, Bethany Bowring, Sasha Jenkins
| 6:00 pm - | WILD WILD WEST INFORMAL EVENING              | Western Development Museum, 2610 Lorne Avenue, Saskatoon<br><br>Sponsored by QIAGEN<br><br>Join us at the WAVLD Dinner Party, sponsored by QIAGEN, at Saskatoon’s unique Western Development Museum. This will be a night that you won’t want to miss! Enjoy great food, refreshments, fun and surprises with lots of opportunity to network as you explore “Boom Town” and experience, “first hand”, Western Canada and Saskatchewan’s history. Purchased ticket required. Shuttle service will be available from downtown hotels to and from the Western Development Museum. |
### Wednesday, June 17, 2015 All events at TCU Place, 35 - 22nd Street East, Saskatoon or otherwise stated.

<table>
<thead>
<tr>
<th>Time</th>
<th>Event</th>
<th>Location</th>
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<tbody>
<tr>
<td>7:30 am - 4:00 pm</td>
<td><strong>REGISTRATION</strong></td>
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<tr>
<td>8:00 am - 9:45 am</td>
<td><strong>PLENARY III:</strong> Detection, Management and Control of New and Emerging Disease</td>
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<td></td>
<td><em>Centennial Hall AB</em></td>
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<td><strong>Sponsored by VMRD</strong></td>
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<td></td>
<td><strong>Emergence of swine enteric coronaviruses in North America</strong></td>
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<td>Richard Hesse, Kansas State Veterinary Diagnostic Laboratory, Kansas State University, USA</td>
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<td>Trying to stay ahead of avian influenza</td>
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<td>Thijs Kuiken, Erasmus University Medical Centre, Rotterdam, The Netherlands</td>
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<tr>
<td>9:30 am - 10:30 am</td>
<td><strong>COFFEE &amp; EXHIBITORS</strong></td>
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<td><strong>POSTERS</strong> - Authors for posters #79 - 93 to be present to answer questions</td>
<td>Grand Salon</td>
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<td>10:30 am - 12:00 pm</td>
<td><strong>CONCURRENT SESSION 5</strong></td>
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<td><em>Centennial Hall AB</em></td>
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<td><strong>Sponsored by Tetracore</strong></td>
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<td>023 Best practices to detect PEDV, TGEV, and PDCoV</td>
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<td><em>Rolf Rauh, William Nelson, Silvia Pillay, Zianab Sankoh, Jane Christopher-Hennings, Travis Clement</em></td>
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<td>024 Real-time PCR reagents for the detection and differentiation of swine enteric coronaviruses</td>
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<td>Martina Kahila, Christa Goodell, Lori Pourde, Kathy Velek, Lisa Gow, Valene Leathers, Michael Angelichio</td>
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<td>025 Validation of the virotype PEDV/TGEV RT-PCR for the detection of PEDV and differentiation from TGEV in recent PEDV outbreaks</td>
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<td><em>Stephen Hennart, Marco Labitzke, Christine Gaunitz, Carsten Schroeder, Oliver Sasse, Claudia Engemann</em></td>
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<td>026 Rapid response to African swine fever: development and validation of QIAGEN’s new virotype ASFV PCR Kit</td>
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<td><em>Daland Christian Herrmann, Christine Gaunitz, Carsten Schroeder, Oliver Sasse, Claudia Engemann</em></td>
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<td>027 Development and validation of a new ASFV real-time PCR</td>
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<td>Elodie Coulon, Sandrine Moine, Immanuel Leifer</td>
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<td>028 Detection of astrovirus in 50-year-old archived tissue samples of cattle with nonsuppurative encephalitis</td>
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<td>Senija Selimovic-Hamza*, Ilias G. Bouzalas, Anna Oevermann, Torsten Seuberlich</td>
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| 8:00 am - 9:30 am | **11TH OIE SEMINAR** New Diagnostic Technologies and International Standard Setting |
|                  | *Gallery CD*                                                          |                           |
|                  | **Welcome and Opening Address**                                       |                           |
|                  | Martine Dubuc, OIE Delegate of Canada                                  |                           |
|                  | **Introduction to the OIE and its Network of Reference Centres, and Projects Linked to New Diagnostic Technologies** |                           |
|                  | Elisabeth Erlacher-Vindel, OIE, Paris, France                         |                           |
|                  | **High Throughput Sequencing, Bioinformatics and Computational Genomics (HTS-BCG): Evolution or Revolution?** |                           |
|                  | Fredrik Granberg, National Veterinary Institute, Uppsala, Sweden      |                           |
|                  | The Increasing Importance of Sequence Information in Managing Animal Health Information Globally: OIE Actions |                           |
|                  | Antonino Caminiti, Istituto Zooprofilattico Sperimentale della Lombardia e Dell’Emilia Romagna, Brescia, Italy |                           |
| 10:15 am - 12:00 pm | **11TH OIE SEMINAR** (continued)                                      |                           |
|                  | **Gallery CD**                                                       |                           |
|                  | Approaches to Validation of High Throughput Sequencing in the Diagnostic Laboratory |                           |
|                  | Ian Gardner, University of Prince Edward Island, Canada               |                           |
|                  | Bioinformatics: How to Standardise and Assemble Raw Data into Sequences. What Does it Mean for a Laboratory to Use Such Technologies? |                           |
|                  | Joseph Hughes, Medical Research Council, University of Glasgow Centre for Virus Research (CVR), United Kingdom |                           |
|                  | New Serotypes/Strains of Bluetongue Virus (BTV) – Animal Health Issues Arising from Reports of Novel BTVs |                           |
|                  | James MacLachlan, University of California, Davis, United States of America |                           |
|                  | General Discussion                                                   |                           |
Wednesday, June 17, 2015  All events at TCU Place, 35 - 22nd Street East, Saskatoon or otherwise stated.

12:00 pm - 1:30 pm  
**LUNCH & EXHIBITORS & POSTERS**  
Grand Salon

1:30 pm - 3:15 pm  
PLENARY IV:  Wild Life and One Health  
Centennial Hall AB  
Sponsored by Svanova  
Diagnostic medicine helps promote ecosystem health in the tropical pacific  
Thierry Work, United States Geological Survey, National Wildlife Health Center, Hawaii, United States  
Disease and conservation biology  
Gary Wobeser, Western College of Veterinary Medicine, University of Saskatchewan, Saskatoon, Canada

1:30 pm - 3:00 pm  
11TH OIE SEMINAR  
Gallery CD  
Quality Assurance of High Throughput Sequencing in the Diagnostic Laboratory  
Gary Van Domselaar, Public Health Agency of Canada  
Tracking Genetic Evolution of Avian Influenza and Related Challenges  
Frank Wong, CSIRO, Australian Animal Health Laboratory, Geelong, Victoria, Australia  
Data Interpretation Challenges: the Example of Genetic and Pathobiological Characterisation of HPAI H5N2 and H5N1 Viruses in Canada  
Yohannes Berhane, National Centre for Foreign Animal Disease, Winnipeg, Manitoba, Canada

3:00 pm - 3:45 pm  
COFFEE & EXHIBITORS  
POSTERS - Authors for posters #94 - 103 to be present to answer questions  
Grand Salon

3:45 pm - 5:15 pm  
CONCURRENT SESSION 6  
Gallery AB  
029 Rabies in African wild-dogs (Lycaon pictus) in northwest South Africa linked to a dog (canid) rabies cycle  
Claude Sabeta, Debra Mohale, Baby Pahladira  
030 Extended-spectrum -lactamase-producing E. coli isolated from wild birds in Saskatoon, Canada, 2014  
Dennilyn Parker, Michelle Sniatynski, Joseph Rubin  
031 Innocuous bat viruses that spillover to other species causing serious disease: differences in human and bat innate antiviral responses  
Arinjay Banerjee*, Noreen Rapin, Vikram Misra  
032 Perception vs reality: Western Canadian equine practitioners’ view of the usefulness of fine needle aspiration in the diagnosis of equine skin disease  
Erin Zachar*, Bruce Wobeser, Hilary Burgess  
033 Serology and molecular diagnostics of Epizootic hemorrhagic disease virus (EHDV)  
Stéphane Daly, Sandrine Moine, Flavia Tisserant, Alex Räber  
034 Comparison between a newly developed ELISA-A/G and some serological tests for the detection of IgG antibodies to Toxoplasma in multiple animal species  
Batol Al-Adhami, Alvin Gajadhar

3:45 pm - 5:00 pm  
11TH OIE SEMINAR  
Gallery CD  
The Challenges of Linking Genetic and Epidemiological Datasets to Reconstructing Transmission Trees for Livestock Viral Diseases  
Donald King, The Pirbright Institute, United Kingdom  
Next Generation Surveillance Systems – Integrating Whole Genome Sequencing Data into Real-Time Disease Detection and Control  
Peter Durr, CSIRO, Australian Animal Health Laboratory, Geelong, Victoria, Australia  
Final Conclusions  
CLOSE

6:00 pm - 10:00 pm  
TASTE OF SASKATCHEWAN BANQUET, PRESENTATIONS, ENTERTAINMENT  
Centennial Hall AB  
Cocktails 6:00 pm - 7:00 pm;  Dinner, Entertainment, Presentations 7:00 pm - 9:00 pm  
Sponsored by the City of Saskatoon  
Join us at the gala banquet sponsored by the City of Saskatoon and featuring a delicious meal of Saskatchewan grown food products. Help congratulate “up and coming” diagnosticians as they are recognized for their achievements. Take the opportunity to relax and visit with “old” and “new” colleagues as you are “wowed” by the entertainment extravaganza featuring top talent from the region. Purchased ticket required. Shuttle service will be available from downtown hotels to and from TCU Place.
### Thursday, June 18, 2015

All events at TCU Place, 35 - 22nd Street East, Saskatoon or otherwise stated.

<table>
<thead>
<tr>
<th>Time</th>
<th>Event Description</th>
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<tbody>
<tr>
<td>7:00 am - 7:45 am</td>
<td><strong>Canadian Animal Health Laboratorians Network (CAHLN) MEETING</strong></td>
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<td>Registration</td>
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<td>8:00 am - 9:45 am</td>
<td><strong>PLENARY V: Turning Laboratory Data into Intelligence</strong></td>
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<td><em>Sponsored by Saskatchewan Ministry of Agriculture</em></td>
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<td>Leverage data for early detection of emerging pathogens</td>
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<td><em>Lina Mur</em>, Institute for Infectious Animal Diseases, Texas, USA</td>
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<td>The role of laboratory data in ‘Knowledgeable Surveillance’</td>
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<td><em>Crawford Revie</em>, Atlantic Veterinary College, University of Prince Edward Island, Charlottetown, Canada</td>
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<tr>
<td>9:45 am - 10:30 am</td>
<td><strong>COFFEE &amp; EXHIBITORS &amp; POSTERS</strong></td>
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<tr>
<td>10:30 am - 12:00 pm</td>
<td><strong>CONCURRENT SESSION 7</strong></td>
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<td><em>Sponsored by Zoetis</em></td>
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<tr>
<td>035</td>
<td>Building animal health surveillance networks using laboratory and field data</td>
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<td><em>Melanie Barham, Bruce McNab, Beverly McEwen, Tim Pasma</em></td>
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<tr>
<td>036</td>
<td>Developing endemic animal disease surveillance capacity using provincial diagnostic animal health labo-</td>
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<td>ratory data</td>
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<td><em>Theresa Burns, Nancy de With, Harold Kloeze, Tyler Stitt, Carl Ribble</em></td>
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<td>037</td>
<td>Vet-LIRN -- A North American network of diagnostic laboratories working with US-FDA to investigate animal</td>
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<td>feed events</td>
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<td><em>Renate Reimschuessel</em></td>
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<td>038</td>
<td>Prairie Diagnostic Services Laboratory Information Management System (LIMS) development experience: a</td>
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<td>case study</td>
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<td><em>Anatoliy Trokhymchuk, Arnie Berg</em></td>
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<td>039</td>
<td>Where to draw the line - determining cut-off thresholds for Lucimenx data using mixture-models</td>
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<td><em>Peter Durr, Victoria Boyd</em></td>
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<td>040</td>
<td>Meaningful sampling in honey bee viral detection</td>
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<td><em>Jennifer Zechel, Hugh Cai, Paul Kozak</em></td>
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<tr>
<td>12:00 pm - 1:30 pm</td>
<td><strong>ITALIAN LUNCH</strong></td>
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<td><em>Sponsored by Thermo Fisher</em></td>
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<td>Join us for wrap-up luncheon following the Thursday scientific sessions featuring a delicious Italian</td>
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<td>menu and café atmosphere that will prime us for the invitation to the WAVLD conference in Italy in 2017!</td>
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<td>Included with registration.</td>
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<td>1:30 pm - 4:30 pm</td>
<td><strong>Canadian Animal Health Surveillance Network (CAHSN) MEETING</strong></td>
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<td><em>Saskatoon's Meewasin River Valley</em></td>
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<td>Take a tour of Saskatoon’s Meewasin River Valley and see our city from the river! Learn some history of</td>
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<td>the area and how the Meewasin Valley Authority, manages parks, cultural amenities, natural grasslands,</td>
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<td>conservation areas and now, River Landing. $25.00.</td>
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<tr>
<td>1:30 pm - 4:30 pm</td>
<td><strong>OFF SITE TOUR - Meet at TCU Place Lobby</strong></td>
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<td>There is limited space available for each tour. Pre-registration is required.</td>
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WORLD ORGANISATION FOR ANIMAL HEALTH
Protecting animals, preserving our future.

11TH OIE SEMINAR
NEW DIAGNOSTIC TECHNOLOGIES AND INTERNATIONAL STANDARD SETTING
to be held during the 17th WAVLD Symposium, Saskatoon (Canada), 15–18 June 2015

PROVISIONAL PROGRAMME

Wednesday 17 June 2015

OPENING
08.00 – 08.10 Welcome and Opening Address
Martine Dubuc, OIE Delegate of Canada

08.10 – 08.30 Introduction to the OIE and its Network of Reference Centres, and Projects Linked to New Diagnostic Technologies; Elisabeth Erlacher-Vindel, OIE, Paris, France

SESSION 1
08.30 – 09.00 High Throughput Sequencing, Bioinformatics and Computational Genomics (HTS-BCG): Evolution or Revolution?; Fredrik Granberg, National Veterinary Institute, Uppsala, Sweden

09.00 – 09.30 The Increasing Importance of Sequence Information in Managing Animal Health Information Globally: OIE Actions; Antonino Caminiti, Istituto Zooprofilattico Sperimentale della Lombardia e Dell’Emilia Romagna, Brescia, Italy

9.30 – 10.15 TEA/COFFEE

10.15 – 10.45 Approaches to Validation of High Throughput Sequencing in the Diagnostic Laboratory; Ian Gardner, University of Prince Edward Island, Canada

10.45 – 11.15 Bioinformatics: How to Standardise and Assemble Raw Data into Sequences. What Does it Mean for a Laboratory to Use Such Technologies?; Joseph Hughes, Medical Research Council, University of Glasgow Centre for Virus Research (CVR), United Kingdom

11.15 – 11.45 New Serotypes/Strains of Bluetongue Virus (BTV) – Animal Health Issues Arising from Reports of Novel BTVs; James MacLachlan, University College Davis, California, United States of America

11.45 – 12.00 General Discussion

12.00 – 13.30 LUNCH

SESSION 2
13.30 – 14.00 Quality Assurance of High Throughput Sequencing in the Diagnostic Laboratory; Gary Van Domselaar, Public Health Agency of Canada

14.00 – 14.30 Tracking Genetic Evolution of Avian Influenza and Related Challenges; Frank Wong, CSIRO, Australian Animal Health Laboratory, Geelong, Victoria, Australia

14.30 – 15.00 Data Interpretation Challenges: the Example of Genetic and Pathobiological Characterisation of HPAI H5N2 and H5N1 Viruses in Canada; Yohannes Berhane, National Centre for Foreign Animal Disease, Winnipeg, Manitoba, Canada

15.00 – 15.45 TEA/COFFEE

15.45 – 16.15 The Challenges of Linking Genetic and Epidemiological Datasets to Reconstructing Transmission Trees for Livestock Viral Diseases; Donald King, The Pirbright Institute, United Kingdom

16.15 – 16.45 Next Generation Surveillance Systems – Integrating Whole Genome Sequencing Data into Real-Time Disease Detection and Control; Peter Durr, CSIRO, Australian Animal Health Laboratory, Geelong, Victoria, Australia

CLOSING
16.45 – 17.00 Final Conclusions
17.00 Close
Conference Information

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The registration desk is located at the TCU Place Lobby Main Floor
Monday 6/15/2015 12:00 PM 8:00 PM Lobby, TCU Place
Tuesday 6/16/2015 7:30 AM 4:00 PM Lobby, TCU Place
Wednesday 6/17/2015 7:30 AM 4:00 PM Lobby, TCU Place
Thursday 6/18/2015 7:30 AM 10:00 AM Lobby, TCU Place

Conference Badges
All delegates will receive the program, conference badges and tickets for the social events (if booked) upon registration at the registration desk at TCU Place, Saskatoon, Canada.

Conference Language
English (no translation available)

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A certificate of attendance will be provided on request.

Meals
Snacks, Lunch and refreshments will be served in TCU Place, Salon A-E.

Poster Presentations
Posters will be presented on numbered panels from Tuesday June 16th until Thursday June 18th.
Special Events

WAVLD Welcome Reception - Monday, June 15 (6:30 PM - 9:00 PM)
Join us at the opening reception where you can meet and greet your colleagues, and warm up for the sessions the following day. Hosted at TCU Place on Monday, June 15 from 6:30 PM - 9:00 PM, the event will feature a selection of complimentary appetizers and refreshments. A special guest speaker will also be featured as part of a short program. This event is included in your conference registration fee.

Wild Wild West Feast - Tuesday, June 16 (6:00 PM - 10:00 PM)
Join us at the WAVLD dinner party, sponsored by Qiagen, at Saskatoon’s unique Western Development Museum. This will be a night that you will not want to miss! Enjoy great food, refreshments, fun, and surprises, with lots of opportunity to network as you explore “Boom Town” and experience Western Canada and Saskatchewan’s history “first hand”. This event is $55.00 per person (accompanying guests welcome).

A Taste of Saskatchewan Banquet - Wednesday, June 17 (6:30 PM - 10:00 PM)
Join us at the gala wrap-up banquet, featuring a delicious meal of Saskatchewan grown food products. Help congratulate “up and coming” diagnosticians as they are recognized for their achievements. Take the opportunity to relax and visit with “old” and “new” colleagues as you are “wowed” by the entertainment extravaganza featuring a taste of Saskatchewan culture. This event is $60.00 per person (accompanying guests welcome).

Wrap-Up Luncheon - Thursday, June 18 (12:00 PM - 1:30 PM)
Join us at the wrap-up luncheon following the Thursday scientific sessions. Sponsored by Thermo Fisher, this luncheon will feature a delicious Italian menu and cafe atmosphere that will prime us for the invitation to the WAVLD conference in Italy in 2017! This event is at no extra cost and included in your conference registration fee.

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Neither the organizers nor the conference office can be held responsible for any liabilities by program changes.

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The City of Saskatoon offers an astonishing array of sites and activities. For further information please visit: http://wavld2015.com/About/VisitingSaskatoon.aspx
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17th International Symposium
of the World Association
of Veterinary Laboratory
Diagnosticians

WAVLD 2015
Plenary:
New Technologies Section
A01 Diagnostics in resource-poor situations: 
a reliable test result even when it is raining and dark

Andrew Soldan

Animal and Plant Health Agency, New Haw, Addlestone, Surrey, UK (andrew.soldan@apha.gsi.gov.uk)

When we move diagnostic testing out of the laboratory, some things don’t change. The quality, cost, and suitability of the test to inform a decision remain vitally important. However, usually the operator is less skilled, many factors become uncontrollable, and the test needs to be very robust.

This presentation will discuss some of the situations where veterinary testing outside the laboratory, in less than ideal conditions, is desirable and highlights factors that need to be taken into consideration when choosing or designing a test. It will give an overview of some exciting technologies that will be available in the next few years for veterinary field and point-of-care testing. Some of these are likely to migrate from the human diagnostic area where there is huge investment in platforms for ‘in clinic’ use. However, many of these platforms, designed for sophisticated primary health care systems, have a high capital and individual use cost. While giving reliable results, they are also unlikely to be robust enough to use in the field.

While these may have future applications in companion animal veterinary practice, it is likely that platforms designed for use in developing world health care systems or for personal use will be more applicable to veterinary field use.
Diagnostic laboratories are expected to recognize when new technologies have reached a tipping point in the balance among client needs, cost, and improved performance. Which of several competing technologies will ultimately prove to be a new gold standard can be difficult to predict. The ultimate arbiter of the choice made should always be the benefit to the client. This has to take into account not only the lab’s estimate of the utility of the new technology but also the successful education of the client on the usefulness, strengths, and weaknesses of the new technology, as well as the correct application of the results generated from it. Several new technologies are approaching that tipping point of implementation, including next-generation sequencing, microfluidics, and metagenomics.

**Next-generation sequencing** (NGS) could combine the wide net of culture methods with the pinpoint accuracy of PCR; to provide both organism identification and functional information, such as antimicrobial resistance or virulence, in one assay.

**Metagenomics** should provide a holistic picture of the microbiome of a patient, providing new insights into the interplay of microorganisms in creating disease. Cost per test, turnaround time, throughput, bioinformatics processing, and result interpretation are still challenges however.

**Microfluidics** is a key element in the creation of a true “lab on a chip” (LOC). Coupled with nanoscale engineering advances, it will substantially impact both cost and portability.

Similar to what occurred with the emigration of hematology, clinical chemistry, and some ELISA testing from the laboratory to the clinic in years past, PCR testing is now poised to make that same journey. Laboratories will need to fill the vacuum left by this shift by adopting new technologies that can only be run in a laboratory setting, as a start. To **remain viable in the future, laboratories must also increase the value of the information generated and adopt a more holistic approach to testing.** The latter will require laboratories to foster close working relationships with a range of subject matter expertise, in epidemiology, medicine, herd health, and management. The goal should be to provide clients not just with individual test results, but rather be part of formulating implementable solutions to their problems, rather than just throwing raw results at them and wishing them good luck.
17th International Symposium of the World Association of Veterinary Laboratory Diagnosticians

WAVLD 2015
Plenary: Control & Management of Endemic Disease
A03 Control and management of endemic disease –
an international Dane’s perspective

Soren Alexandersen

Dugald, Manitoba, Canada (soren.alexandersen@yahoo.ca)

The tools, knowledge, coordination, and infrastructure needed to control endemic diseases are briefly summarized. However, it is also emphasized, that albeit the details of control may be somewhat different for each infection, overall control and management is independent of an infection being endemic or a foreign animal disease (FAD) incursion. Moreover, an FAD in one country or part of the world may be endemic in another. Importantly, the will and commitment to control, manage, or eradicate any infection need to be strong, and the full path to the end game envisioned ahead of time.

Various examples of specific disease control/eradication programs are given, including some successes and at least one failure.
A04 Molecular evolution and the consequential diagnostic challenges for infectious bursal disease viruses.

**Daral J. Jackwood**

*Ohio Agricultural Research and Development Center, The Ohio State University, Wooster, Ohio, USA (jackwood.2@osu.edu)*

Infected is a member of the family Birnaviridae, genus Avibirnavirus. The serotype 1 viruses typically infect chickens and cause immune suppression.

IBDV serotype 1 pathotypes have been classified into **sub-clinical (scIBDV), classic virulent (cvIBDV), and very virulent (vvIBDV) groups.** All 3 pathotypes cause immunosuppression in chickens, but the vvIBDV also cause high mortality. Phylogenetic studies indicate vvIBDV have spread to most poultry-producing countries in the world, including the United States. However, they have not yet been found in Canada.

Because numerous antigenic types of serotype 1 IBDV exist, detection assays strive to identify the antigenic subtype of these viruses so they can be matched to a homologous vaccine strain. The only reliable assay that can distinguish antigenic differences among IBDV is in vitro virus-neutralization. Because these are expensive assays and most IBDV do not replicate in vitro, identification of IBDV has moved away from classical virology and toward molecular techniques. **Most laboratories use RT-PCR followed by nucleotide sequencing of the gene encoding the antigenic VP2 capsid protein.** Although some point mutations in VP2 are known to contribute to antigenic drift, the molecular basis for antigenicity is not fully understood. In addition to point mutations, molecular diversity among the IBDV can result from homologous recombination. Thus, interpretation and correlation of sequence data with antigenicity can be precarious.

Using molecular sequencing to predict pathogenicity and identify vvIBDV has also been difficult. Since VP1, encoded by genome segment B, partially controls pathogenicity, molecular detection of vvIBDV requires the inclusion of both genome segments in the sequence analysis. Reassorting of the 2 genome segments has produced viruses with a vvIBDV genome segment A and a non-vvIBDV genome segment B. These viruses are less virulent than IBDV with both vvIBDV genome segments.

Using molecular sequence data to identify the different strains of IBDV is still the best option for routine detection, but genetic variability observed among the viruses can make it difficult to interpret the results.
17th International Symposium of the World Association of Veterinary Laboratory Diagnosticians

WAVLD 2015
Plenary:
Detection, Management and Control of New and Emerging Disease
A05 Emerging swine enteric coronaviruses in North America

Richard Hesse

Kansas State Veterinary Diagnostic Laboratory, Kansas State University, Manhattan, KS, USA (dhesse@vet.k-state.edu)

Porcine epidemic diarrhea virus (PEDV) and Porcine deltacoronavirus (PDCoV) are newly emerged swine enteric coronaviruses in North America. PEDV was first observed in the United States in April of 2013 and PDCoV in November of 2013. Since then, both have spread across the western hemisphere killing an estimated 8-10 million pigs. Experimental animal inoculation studies for both viruses have recently been conducted; data from these studies will be presented.

The first study investigates the tissue localization, shedding, virus carriage, antibody response, and aerosol transmission of PEDV following inoculation of 4-week-old feeder pigs. Oral-nasal inoculation results demonstrated that pigs developed mild to moderate clinical diarrhea at 2 days post-inoculation (DPI) that resolved by 8 DPI. Fecal and nasal swabs were PCR-positive for the virus at 48 hours post-inoculation and maintained that positivity in some animals up to 35 DPI. Interestingly, small intestinal homogenates from 70% of group A pigs were PCR-positive at 42 DPI. Viremia was detected in ~50% of animals that developed clinical disease. Inoculated pigs and contact controls all seroconverted to the virus by 14 DPI. Immunohistochemistry (IHC) demonstrated the presence of viral antigen in the small intestine and occasional mesenteric lymph nodes of sequentially euthanized pigs. Aerosol transmission did not occur in this study as demonstrated by lack of PCR detection in fecal swabs and lack of seroconversion.

The second study investigated the role of PDCoV as a primary pathogen of nursing pigs and their dams as well as looking at tissue localization, shedding, virus carriage, antibody response, and aerosol transmission. Sows and pigs were oral-nasally inoculated with a PDCoV inoculum from a field sample (negative for other enteric disease agents), when the pigs were 2-4 days of age. Serial samples of blood, serum, oral fluids, nasal and fecal swabs, and tissues from sequential postmortems were collected throughout the 42-day study for PCR, histopathology, and IHC. Diarrhea developed in all inoculated and contact control pigs, including sows, by 2 days post-inoculation (DPI) and aerosol control pigs by 3-4 DPI. All animals were clinically normal by 12 DPI. Atrophic enteritis was observed in the jejunum and ileum of affected piglets from 2 to 8 DPI, and matched with IHC detection of viral antigen. Mesenteric lymph node and small intestine were the primary sites of antigen detection by IHC and tissue PCR. Most pigs and sows were PCR-positive in these tissue samples out to the end of the study. In contrast, fecal and nasal shedding was observed in some animals to 21 DPI. This study demonstrates the time course of pathogenicity and virus kinetics of PDCoV in conventional neonatal and adult swine.
Influenza A virus infections, originating in wild waterbirds, are capable of switching hosts to wild mammals, domestic animals, and people. Rapid global changes, including burgeoning poultry and swine populations, are associated with unusual host switches of particular influenza A virus strains, some with fatal consequences. Both highly pathogenic avian influenza virus (HPAIV) H5N1 and low pathogenic avian influenza virus (LPAIV) H7N9 may be transmitted to people due to direct or indirect exposure to infected live or dead poultry or contaminated environments. Between 2003 and February 2015, laboratory-confirmed HPAIV H5N1 infections have been reported in 718 people, of whom 413 have died; and between 2013 and February 2015, laboratory-confirmed LPAIV H7N9 infections have been reported in 486 people, of whom 185 have died.

HPAIV H5N8 appears to have acquired the ability to spread efficiently in wild bird populations. After emerging in poultry in Asia (Korea, Japan) in early 2014, HPAIV H5N8 spread to wild birds and poultry in Europe (Germany, the Netherlands, the UK, and Italy) and North America (Canada and the USA) in November and December 2014.

In this presentation, I will review viral adaptations that allow such host switches. Also, I will discuss possible improvements in surveillance, detection, and management to mitigate the burden of disease from influenza.
17th International Symposium of the World Association of Veterinary Laboratory Diagnosticians

WAVLD 2015
Plenary: Wildlife and One Health
Tropical coral reefs are known, with justification, as the rainforests of the sea. They are some of the most diverse ecosystems on earth providing a variety of environmental and other economic services to human communities globally. Unfortunately, these tropical marine ecosystems face numerous threats. Terrestrial runoff from coastal developments smothers corals leading to mortality. Warming temperatures exacerbate bleaching events in which corals lose their symbiotic algae causing them to lose their colors and turn white. Ocean acidification is already impairing the ability of many marine organisms to deposit calcium, and acidification’s effect on corals is potentially dire. Overfishing is also imposing increasing burdens on coral reefs because of disturbances to ecosystem structure. Finally, disease is playing an increasingly important role in decline of coral reefs. For example, in the Western Atlantic, corals have declined by 80%, mainly because of disease. Yet, in spite of over 40 years of research, our knowledge of coral anatomy, physiology, and causes of disease in corals is rudimentary. The main reason for this is that standard biomedical approaches used to investigate diseases in other animals have not been systematically applied to coral reefs, and we have little idea of what occurs at the cellular and tissue level in coral disease.

Veterinary diagnosticians, with their knowledge of comparative medicine, are poised to potentially play an important role in furthering our understanding of causes of and pathogenesis of disease in this important group of animals. Specifically, there is a need for diagnostic pathologists to play a central role, because this discipline provides a logical framework to guide subsequent laboratory confirmations, gives concrete evidence at the cellular level of potential causes of disease and host response, and serves as a reference point to relate experimental studies to disease in the wild. By applying basic histopathology judiciously supplemented by molecular and other approaches, veterinary diagnosticians working jointly with coral ecologists could develop novel approaches and make exciting new discoveries. Unlike other wildlife disease systems where the loss of a species seldom leads to massive ecological changes, corals, as ecosystem engineers, are the environment, and their loss has profound ecosystem implications. By helping to understand causes of coral disease and proposing management options based on their findings, veterinary diagnosticians could make significant contributions not only to wildlife health but conservation of marine ecosystems.
A08 Disease and conservation biology

Gary A. Wobeser

Department of Veterinary Pathology, Western College of Veterinary Medicine, University of Saskatchewan, Saskatoon, Saskatchewan, Canada (gary.wobeser@usask.ca)
17th International Symposium of the World Association of Veterinary Laboratory Diagnosticians

WAVLD 2015
Plenary: Turning Laboratory Data into Intelligence
Previous FAD outbreaks have clearly demonstrated that any delay in disease detection results in an enormous increase in economic and other associated negative consequences. For this reason, early detection and rapid control of animal diseases is the most critical concern of veterinary services. In order to achieve this, alternative approaches and new technologies and tools are acquiring great importance for their implementation, including risk-based methods, syndromic and digital surveillance, and innovative information science, among others. These approaches rely on the collection of appropriate information that would allow earlier detection of emerging diseases (as compared to traditional methods) and/or a rapid, more efficient control of disease outbreaks and decision-making. This information can be obtained not only by leveraging the health-related information that currently exists in data records and is commonly collected, but also collecting and integrating other data sources either not currently being recorded or available to decision-makers for use. However, the process of collecting and analyzing these data to provide meaningful information can be a difficult, non-standardized, and complex procedure. A review of the most important sources of information that can be leveraged for animal health management, and the limitations, challenges, and problems encountered when collecting and analyzing different sources of data will be discussed.

In this context, clinical data has been pointed out as the data source able to provide information on animal health in the earliest stage. Other real-time animal monitoring tools (such as thermography or accelerometers) have demonstrated their utility in detecting individual animal changes; however, the feasibility, representativeness, and scalability of these tools for broader implementation should be studied. On the other hand, there are numerous sources containing data directly related with health problems. Laboratory data is probably the most important one and the most studied in the veterinary field. Still, there are many other sources, such as animal movement data, market prices, and phylogenetic data that can be utilized to enhance animal health and inform decision-makers.

The use of disparate data sources brings with it additional challenges in terms of data harmonization, standardization, analysis and evaluation, as each data source may require different preprocessing, integration, and analytical methods for obtaining the maximum profit. Overcoming these constraints is imperative, as the information obtained from combining all these data sources is vital for the early detection of emerging pathogens.
A10 The role of laboratory data in ‘knowledgeable surveillance’

Crawford W. Revie, Fernanda Dórea

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Over the past decade, the availability of digital data related to animal health has grown exponentially, and with it an interest in making effective and timely use of these data. In particular, the use of syndrome-based indicators to augment traditional laboratory results for the purpose of disease surveillance has been the focus of a number of studies. The volume and semi-structured nature of such data, together with the fact that it must often be processed in real time, have led to methodological challenges in the appropriate interpretation of these novel data sources. In this talk, we will discuss a range of techniques ranging from **text-mining, times-series analyses, and clustering algorithms** that can be used to identify syndromic signals in laboratory test request data, together with **statistical techniques** that can be used to detect the various types of temporal aberrations that can occur. These approaches have been implemented in systems linked to animal health laboratory systems in Canada and Sweden, and their use will be illustrated by way of case-based examples.

However, the isolated use of laboratory data is rarely adequate in the context of syndromic surveillance, and a variety of animal health data sources are being explored for early disease detection. In terms of ‘next steps’ towards successfully using such data, we believe that the integration of evidence from multiple sources is of critical importance. A key challenge in moving forward is the need to ensure that aggregation and comparison across data sources is being made among similar objects. In this context, we are exploring the use of **knowledge-based ontologies**, which provide machine-readable methods for the representation of and inference from data. We will discuss one such pilot ontology – AHSO (Animal Health Surveillance Ontology) – and illustrate the ways in which the availability of frameworks such as this can be complemented by recent advances in computer science, including **deep learning** and the **Semantic Web**.

Research results from these areas will allow for the integration of information derived from diagnostic data with that extracted from other sources of animal health information, including clinical records, mortality, and even regular production data, to create a framework for truly “knowledgeable” surveillance.
17th International Symposium of the World Association of Veterinary Laboratory Diagnosticians

WAVLD 2015
Abstracts: Oral
Rapid and sensitive detection of Classical swine fever virus on a portable compact user-friendly device with automatic display of results

Oliver Lung, John Pasick, Mathew Fisher, Cody Buchanan, Anthony Erickson, Aruna Ambagala

Canadian Food Inspection Agency, Lethbridge, AB, (Lung oliver.lung@inspection.gc.ca; Fisher, Buchanan, Erickson, Ambagala); and Winnipeg, Manitoba, Canada (Pasick)

Classical swine fever (CSF) is a highly infectious disease that can have a severe economic impact on the swine industry. The causative agent, Classical swine fever virus (CSFV) is a member of the genus Pestivirus of the family Flaviviridae. The clinical signs of CSF can vary from mild to severe in domestic and wild pigs. The most common clinical signs include fever, hemorrhages, anorexia, ataxia, diarrhea and pneumonia due to opportunistic bacterial infections.

User-friendly and sensitive diagnostic tests that utilize compact field-deployable instruments for rapid CSF diagnosis can be useful for disease surveillance and outbreak monitoring. In this study, we describe the evaluation of a simple, probe-based, insulated isothermal reverse transcriptase PCR (iiRT-PCR) assay that uses lyophilized reagents for rapid detection of CSFV. The user-friendly assay was conducted on a compact device that automatically displays the results as “+” or “-” after each run. The CSF iiRT-PCR assay accurately detected RNA from a diverse panel of 34 CSFV strains representing all 3 genotypes (n=33) and RNA transcribed from a cloned sequence. No detection was observed with a panel of 18 non-CSF viruses including eight other pestivirus strains representing bovine viral diarrhea virus type 1 and type 2, border disease virus, HoBi atypical pestivirus, and 10 other viruses associated with livestock (African swine fever virus, swine vesicular disease virus, swine influenza virus, porcine respiratory and reproductive syndrome virus, porcine circovirus 1, porcine circovirus 2, porcine respiratory coronavirus, vesicular exanthema of swine virus, bovine herpesvirus 1, and vesicular stomatitis virus). The assay detected CSFV as early as 2 days post-inoculation in RNA extracted from serum samples of experimentally infected pigs. The analytical sensitivity of the assay determined using in vitro transcribed RNA was approximately 23 copies per reaction. The turnaround time from sample to result is one hour using a low volume of unprocessed neat serum, or diluted serum, or extracted RNA.

A user-friendly, portable device that uses lyophilized reagents and automatically analyses and displays results could be a useful tool for surveillance and monitoring of CSF on-site and in the laboratory.
Detection of bovine and equine rotaviruses by reverse transcription-insulated isothermal polymerase chain reaction on a field-deployable device

Rebecca Wilkes, Mohamed Soltan, Yun-Long Tsai, Chuan-Fu Tsai, Hsiu-Hui Chang, Pei-Yu Lee, Hsiao-Fen Chang, Hwa-Tang Wang

Biomedical and Diagnostic Sciences, University of Tennessee, Knoxville, Tennessee, USA (Wilkes beckpen@utk.edu ; Soltan); GeneReach Biotechnology Corp., Taichung, Taiwan (Y-L Tsai, C-F Tsai, H-H Chang, Lee, H-F Chang, Wang).

Rotaviruses are important pathogens in neonatal animals. Insulated isothermal polymerase chain reaction (iiPCR) with POCKIT™, a field-deployable system, is an easy-to-perform molecular tool for sensitive and specific pathogen detection (1,2). The aims of this study were to evaluate the applicability of a rapid and user-friendly rotavirus reverse transcription (RT)-iiPCR in detecting rotavirus infection in cattle and horses.

An RT-iiPCR targeting the nsp5 gene of all bovine and equine rotaviruses was developed, generating a 94-bp amplicon. The RT-iiPCR did not react with other causes of diarrhea in horses and cattle, including Neorickettsia risticii (Potomac horse fever), Rhodococcus equi, Clostridium difficile, Escherichia coli, Salmonella enterica Typhimurium, bovine viral diarrhea virus genotypes I and II, bovine coronavirus, and Cryptosporidium sp. The limit of detection 95% (confidence interval) was determined by probit analysis to be 4 and 3 copies of dsRNA, respectively, using log dilutions of in vitro transcribed dsRNA containing target bovine and equine rotavirus sequences. Performance of the rotavirus RT-iiPCR was further evaluated by comparison with a commercially available real-time RT-PCR (qRT-PCR) reagent (LSI VetMAX™ Triplex Ruminant Rotavirus & Coronavirus Real-Time PCR Kit, Life Technologies). Testing serial dilutions of a clinical isolate of bovine rotavirus showed that RT-iiPCR had a 100-fold increase in sensitivity compared to the qRT-PCR. Furthermore, side-by-side test results of bovine (n=76) and equine (n=22) clinical fecal samples showed that RT-iiPCR failed to detect 2 (one bovine, one equine) of the 54 (51 bovine, 3 equine) qRT-PCR-positive samples and reacted positively with 5 (all bovine) of the 44 (25 bovine, 19 equine) qRT-PCR-negative samples. Performance of the RT-iiPCR had a negative percent agreement of 88.64% (confidence index 95%: 78.24-99.03%) and a positive percent agreement of 96.30% (confidence index 95%: 89.59-100%) with qRT-PCR. Accordingly, the total agreement was 92.86% (kappa =0.85).

The relatively low negative percentage agreement between RT-iiPCR and reference qRT-PCR was likely due to the sensitivity difference between the 2 assays. In conclusion, the user-friendly field-deployable RT-iiPCR-POCKIT™ system holds substantial promise for on-site veterinary diagnosis of rotavirus infection.

References
A novel molecular diagnostic tool for Equine arteritis virus detection and characterization

Delphine Gaudaire, Nicolas Berthet, Aymeric Hans

Equine arteritis virus (EAV) is a member of the Arteriviridae family. EAV infects equids and can persist in the reproductive tract of stallions only. Persistently infected stallions shed the virus in their semen and spread the virus in the horse population during breeding. Moreover, infection may cause abortion in pregnant mares and the death of young foals. OIE prescribes viral isolation (VI) on cell culture to detect EAV for international trade. However, a recent study showed that the real-time reverse transcription-PCR (RT-qPCR) assay developed in 2002 (1) is as sensitive as VI for detecting EAV in semen (2). The main challenge to EAV surveillance is detecting EAV to prevent costly outbreaks, considering in particular the limited number of viral nucleotides targeted in some samples. The aim of our study was to increase the sensitivity of the OIE-recommended RT-qPCR method by combining it with an unbiased amplification method using the Phi29 polymerase coupled to a high-density resequencing microarray (RMA) to genotype the viruses detected. Sixty different samples were used in this study. Of the 48 EAV-positive samples, 31 were from semen, 12 were from virus isolation cell culture supernatants, and 5 were tissue samples from the lungs, spleen or liver of one aborted foetus, 3 young foals, and an adult. Our results showed that isothermal amplification polymerase significantly increased the ratio of amplification from a factor ranging from 10^2 to 10^7 compared to the OIE-recommended RT-qPCR method (3). To genotype the viruses detected, we combined the unbiased amplification of nucleic acids with a RMA. The 2 EAV sequences tiled on the microarray cover a region located in ORF 1 coding for the non-structural protein 9. Surprisingly, the phylogenetic tree obtained with the nsp 9 nucleotide sequences retrieved from the microarray was able to separate strains into the NA and EU groups and divided the EU group into subgroups EU-1 and EU-2 (3).

In conclusion, this method can be recommended for the detection of EAV in semen and aborted fetuses, especially when viral load is very low. In addition, this study confirmed and validated the usefulness of the high-density resequencing DNA microarray for both the diagnosis of equine viral diseases and the genotyping of RNA viruses such as equine arteritis virus.

References

Solutions for rapid detection of pathogen nucleic acids in liquid animal samples: introducing QIAGEN's new cador MagBead Kit

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An effective, robust sample preparation method ensuring the reliable purification of DNA and RNA is key to the successful identification of pathogen nucleic acids. Processing different samples in parallel – including multiple pathogens and starting materials – with the same protocol enables a streamlined workflow for veterinary pathogen detection, saving time and effort. Furthermore, expanded throughput options increase flexibility when processing a large number of samples. With the new MagAttract 96 cador Pathogen Kit (cador MagBead Kit) using magnetic bead technology and the cador Pathogen 96 QIAcube HT Kit using silica membrane-based spin-column technology, QIAGEN has developed 2 sample preparation kit solutions specially designed for fully automated use in a 96-well format. Both kits can be used for the parallel processing of different sample types and for the co-extraction of viral RNA and DNA and bacterial DNA.

In an internal validation study, various sample material such as whole blood, serum, oral fluid, and homogenized tissue were tested for different RNA viruses such as Bovine viral diarrhea virus (BVDV), Porcine reproductive and respiratory syndrome virus (PRRSV), Influenza A virus, Schmallenberg virus (SBV), and Classical swine fever virus (CSFV). Sample extraction was performed in parallel with the new cador MagBead Kit and the cador Pathogen 96 QIAcube HT Kit, and compared to proven manual extraction methods. Purified RNA was then analyzed by real-time RT-PCR using virotype RT-PCR Kits (QIAGEN).

The internal study revealed comparable real-time RT-PCR results for both automated sample preparation kits. Depending on the sample material and pathogen, the results varied from slightly lower mean Ct values to somewhat higher mean Ct values. For serum samples, an average Ct deviation ranging from 0.26 to 1.4 was measured using the cador MagBead Kit. Testing BVDV and PRRSV positive serum samples extracted with the cador MagBead Kit showed slightly better RT-PCR results compared to another commercially available magnetic bead kit. Furthermore, external testing was performed by using the new cador MagBead Kit in different veterinary diagnostic labs. The external validation study showed mostly better results when using the new cador MagBead Kit for the extraction of PRRSV, influenza A virus, and bacteria-positive samples.

QIAGEN provides a new magnetic bead automated processing solution for rapid automated purification of both viral RNA and DNA, as well as bacterial DNA from a variety of different animal samples. The new cador MagBead Kit is all-inclusive, containing all the necessary plasticware. This allows fast and reliable processing of 96 samples per run using the same universal protocol. The cador Pathogen 96 QIAcube HT Kit can also be used for parallel processing of different sample types in one run. Both extraction kits enable robust, reproducible purification of nucleic acids ready for analysis by real-time PCR/RT-PCR.
Performance evaluation of the Fluidigm BioMark™ platform for high-throughput microbe monitoring in salmon

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A formal evaluation of the Fluidigm BioMark™ high-throughput microfluidics platform for use in microbe surveillance research was undertaken. Forty-seven assays to 46 microbes (including 4 OIE-listed viruses) suspected or known to cause disease in salmon worldwide were developed or derived from the literature, and appropriate controls obtained. The evaluation completed stage-1 of the OIE pathway, ascertaining the analytical sensitivity, specificity, and repeatability, and preliminary reproducibility (in which we assessed comparability between 2 PCR platforms within a laboratory rather than within a platform between laboratories) for all 47 assays. The microfluidics platform can assess duplicates of all 47 microbes at once, but to do so, due to the very small size of the reaction chambers (7 nL), requires an enrichment step that includes a multiplex with all assay primers. Therefore, we additionally assessed the role of this specific target amplification (STA) enrichment step on the specificity and relative quantitation of the assays.

This study included over 300,000 qPCR reactions from 36 (96.96) BioMark dynamic arrays and 12 (384-well) ABI 7900 plates. The assays evaluated on the BioMark platform performed to high standards, demonstrating a high degree of sensitivity (limit of detection 1-10 copies per reaction chamber), specificity (most assays demonstrating >98% sensitivity and specificity to the targeted microbes), and repeatability (averaging 96% over all assays for samples run by 2 technicians and near perfect agreement for scoring alone). Consistency of data between the BioMark and ABI 7900 platforms was also high, with a concordance correlation coefficient after correction for the 8-10 Ct difference between platforms of 0.95. If samples were only scored positive for detections in duplicate samples and appropriate Ct cutpoints were applied, specificity and sensitivity between platforms rose to >0.99. Finally, there was no evidence that the STA enrichment step had a substantial impact on the sensitivity or specificity of the assays.

Overall, the BioMark platform provided reliable, rapid and inexpensive quantitative data on presence and load of most microbes. We identified a few assays (Candidatus Branchiomonas cysticola, infectious salmon anemia virus (segment 7), Paranucleospora theridion, Piscichlamydia salmonis, piscine myocarditis virus, and viral hemorrhagic septicemia virus) that performed more poorly than others on one or more measures, and one assay that will be removed (Yersinia ruckeri). We did not evaluate the platform for diagnostic use, but given its positive performance and ability to assess dozens of microbes at once at a considerable cost and time savings, diagnostic laboratories should consider adopting this platform for microbe surveillance.
006 Investigation of shedding pattern of fowl adenovirus (FAdV) following live FAdV vaccine in broiler breeders

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Fecal shedding of fowl adenoviruses (FAdV) has been demonstrated in specific pathogen free (SPF) white leghorn chickens. However, no information is available regarding FAdV shedding and the effect of serum neutralizing antibodies (NAb) on FAdV shedding in broiler breeders. The objectives of the study were to investigate fecal shedding of FAdV following vaccination of broiler breeders with a live FAdV vaccine and to study effect of serum NAb on FAdV shedding.

Naïve broiler breeders were vaccinated orally at 17 weeks of age or 49 weeks of age with FAdV8ab (1x10^4 TCID$_{50}$/bird). In the first experiment at the 17th week, half of the birds (n=12) were vaccinated and the remaining half (n=12) of the birds were housed together with vaccinated birds. Another group (n=24) of birds was kept as unvaccinated control. Cloacal swabs (n=3 to 5) were collected weekly at 16 through 23 and 31, 33, 36, 40, 43 weeks of age, and sera (n= 9 to 11) were collected at 16, 23, 33, and 48 weeks of age. In a crossover trial, 22 broiler breeders were vaccinated at 49th week. Cloacal swabs (n=13 to 22) were collected weekly at 50 through 60 and at 63 weeks of age. Serum samples (n=13 to 22) were collected at 49, 51, 52, 53, 55, and 63 weeks of age. Fecal shedding was determined by qPCR (FAdV-DNA) and NAb were measured by a serum neutralization assay.

Broiler breeders shed infectious virus in feces for 7-21 days post-infection (dpi) that peaked at 7dpi, and disappeared after 21 dpi. FAdV shedding was similar in both experiments and in non-vaccinated broiler breeders housed with vaccinated birds. NAb titer was negatively correlated with FAdV shedding. This study demonstrates the effect of NAb on shedding of FAdV in broiler breeders vaccinated with a live FAdV vaccine.

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Efficacy of commercial infectious bursal disease (IBD) vaccines against “variant IBD virus-NC171” infection in broiler chickens

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The immune suppression that results from variant strains of infectious bursal disease virus (vIBDV) infections has major economic impact on the broiler chicken industry in Canada. In a previous study, we demonstrated that current broiler breeder vaccines are not effective against vIBDV in broiler chickens. This study was conducted to determine the efficacy of commercially available IBDV broiler vaccines against vIBDV in broilers.

A group of broiler hatching eggs was vaccinated, at day 18 of incubation, with a turkey herpesvirus-infectious bursal disease (HVT-IBDV) vector vaccine by in ovo route. Another group, consisting of day-old broiler chickens, was vaccinated with a commercially available live-attenuated IBDV vaccine by subcutaneous route. All the chickens of vaccinated and non-vaccinated (control) groups received 3 X 10^3 EID50 of variant IBDV strain NC171 at day 6 post-hatch. A randomly selected sub-set of each group (n=20) were sampled at days 19 and 35 post-hatch for bursal weight to body weight percentage (BBW) and bursal histopathology. A quantitative real time RT-PCR assay was used to detect the IBD viral genome and viral load by cycle threshold values in the bursa tissues at 9, 12, 6, 15, 19, 24, 29, 35 days of age following IBDV challenge at 6 days of age. vIBDV-NC171 was re-isolated by inoculating bursal tissue homogenate into specific pathogen free eggs, and embryo lesions were observed. A parallel similar trial was conducted using broiler progenies obtained from broiler breeders experimentally vaccinated with live vIBDV (single dose, 3 X 10^3 EID50/bird) isolated in Canada.

The groups of broilers that received either HVT-IBDV or live-attenuated vaccine had significantly lower BBW and severe bursal atrophy compared to broilers from parents experimentally vaccinated with vIBDV NC171 or 05SA8 (P<0.05). Broilers from parents vaccinated against vIBDV NC171 or 05SA8 had very low IBDV load compared to broilers vaccinated with commercial broiler vaccines. It is evident that immunity induced by these commercial broiler vaccines was not able to control vIBDV infection in broilers.
Oligodeoxynucleotides containing CpG motifs (CpG-ODN) as an immunoprotective agent against viral diseases in broiler chickens

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Synthetic oligodeoxynucleotides (ODN) containing CpG motifs (CpG-ODN) have been proven as agents that can activate host defense mechanisms leading to stimulation of the innate immune system against a range of bacterial, viral, and protozoal infections in many vertebrate species. We have previously shown that in ovo delivery of CpG-ODN were able to significantly protect neonatal broiler chickens against *Escherichia coli* or *Salmonella* Typhimurium infections. CpG-ODNs have also been used as an adjuvant against viral infections. The objective of this study was to identify the immune-protective effects of CpG-ODN against economically important viral diseases in broiler chickens.

To study the immune-protective effects of CpG-ODN against variant infectious bursal disease virus (vIBDV), a group of 50 neonatal broiler chickens were given either CpG-ODN (50µg/bird) or saline by the intramuscular route at 3 days of age. All birds were then challenged with vIBDV, NC171 (3 X 10^3 EID<sub>50</sub>/bird) at 6 days of age. The bursae of Fabricius (n=3) were collected and vIBDV load quantified by RT-PCR at 8 time points (14 h, 23 h, 2 d, 3 d, 5 d, 7 d, 13 d, 29 d) following vIBDV challenge. No vIBDV was detected up to day 7 post-challenge in the group that received CpG-ODN. In contrast, vIBDV was detected as early as 14 hours post-challenge in the saline group. A second experiment used 50 birds with no maternal antibodies against fowl adenoviruses that were given either multiple doses of CpG-ODN (50 µg/bird) or saline at 12, 14, 16, and 18 days of age. Birds were challenged with FAdV 11(10^6 TCID/bird) at 14 days of age. We observed statistically significant protection against FAdV 11 following multiple doses of CpG-ODN.

In conclusion, CpG-ODN showed protective effects on viral disease in chickens, however; confirmation of the mechanisms of these effects by further detailed studies are needed.
Development and evaluation of a multiplex molecular assay for poultry pathogens with lab-on-chip

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Poultry pathogens have significant impact on animal health, food safety and public health. It is therefore critical to develop rapid and sensitive assays for accurate diagnosis and effective disease investigation.

A microfluidic lab-on-chip integrating multiplex molecular amplification and DNA microarray hybridization was developed for simultaneous detection of 12 poultry pathogen targets, including avian influenza virus (AIV) matrix gene, avian influenza virus HA gene for H5, H7 and H9, avian influenza virus NA gene for N1, Newcastle disease virus (NDV) matrix gene, Newcastle disease virus fusion gene, Salmonella spp, SalmonellaEnteritidis, Salmonella Pullorum, Chlamydophila psittaci, and Campylobacter spp.

The analytical performance of the lab-on-chip for each pathogen target ranged from $10^2$ to $10^3$ DNA or RNA copies. Assay performance was further verified with field samples spiked with viral and bacterial isolates. This lab-on-chip was subsequently assessed and evaluated in our own Animal Health Laboratory and at the Australia Animal Health Laboratory, Geelong, using 178 poultry viral and bacterial samples. The lab-on-chip had a sensitivity of 94.9% and a specificity of 100% for avian influenza; a sensitivity of 86.7% and a specificity of 99.2% for avian influenza H5 strains; a sensitivity of 76.9% and a specificity of 98.4% for avian influenza H7 strains; and a sensitivity of 80.0% and a specificity of 99.2% for influenza H9 strains; a sensitivity of 76.9% and a specificity of 100% for avian influenza N1 strains; a sensitivity of 80% and a specificity of 100% for Newcastle disease virus matrix gene; a sensitivity of 6.7% and a specificity of 100% for NDV fusion gene; a sensitivity of 64.7% and a specificity of 100% for Salmonella spp; a sensitivity of 100% and a specificity of 99.34% for Salmonella Enteritidis; a sensitivity of 100% and a specificity of 100% for Salmonella Pullorum; a sensitivity of 50% and a specificity of 100% for Chlamydophila psittaci; and a sensitivity of 100% and a specificity of 97.4% for Campylobacter spp. Importantly, the lab-on-chip successfully detected emerging AIV H5 clades such as 2.3.2.1a and 2.3.2.1c. Results suggested the practicality of an amplification and microarray-based approach in a field setting for early detection and identification of poultry pathogens.

Further optimization for detection of NDV fusion gene, Salmonella spp. and Chlamydophila psittaci is ongoing, which may involve modifications to primer and probe sequences. Also further optimization can improve the assay for detection of influenza, assay sensitivity. Experience gained in the project could facilitate the development of multiplex molecular diagnostic assays for animal and human pathogens in general.
Identification of bacterial species isolated from non-viable chicken embryos in Western Canadian hatcheries

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During the last few years, yolk sac infections have been increasing in broiler chickens in Western Canada, leading to significant economic losses for the poultry industry. Bacterial contamination of hatching eggs due to hatchery and broiler breeder management issues may be associated with early yolk sac infections and subsequent embryonic death. The objective of this study was to identify potential emerging bacterial agent(s) isolated from dead chicken embryos during the egg incubation period.

Hatch debris, which is unhatched eggs at various stages of embryonic development during 21 days of incubation, was collected and analyzed from 3 commercial hatcheries across Western Canada, over a period of one year. We examined 3,487 hatch debris for stages of embryonic death and have observed that the majority of embryo mortality (n=1,871; 53.65%) occurred during the early stage; 1-7 days (19.25%) and late stage; 17-21 days (34.40%) of incubation. Randomly selected yolk samples (n=750; 250 per hatchery) from early and late stages dead embryos were cultured on sheep blood agar (Oxoid, Nepean, Ontario, Canada) and incubated under aerobic and anaerobic environment at 37°C for 48 h. Bacterial identification was conducted using the matrix assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS) technology.

Bacterial growth was obtained from 494 (66%) samples, whereas 256 (34%) had no growth. A total of 920 isolates were recovered in pure or mixed cultures from the dead embryo samples with Enterococcus sp. being the most frequently isolated organism, accounting for 331 isolates (36%), followed by Escherichia coli, 207 isolates (22.50%). Of the Enterococcus sp. isolates, 271 (81.87%) were identified as E. faecalis, followed by E. faecium, 30 (9.06%). The remaining isolates included Staphylococcus spp., Streptococcus spp., Pseudomonas spp. and other enterobacteria. Overall, MALDI-TOF MS was able to identify 83.70% of isolates to the genus level and 71.30% to the species level. The stages of embryonic mortality and bacterial species identified were similar in all 3 hatcheries. It is well established that E. coli is associated with yolk sac infection leading to embryonic death; however, isolation of high numbers of Enterococcus species from dead chicken embryos suggests that this organism may be considered a significant and emerging pathogen in the poultry industry. In fact, for the last few years, Enterococcus species have been isolated from a significant number of yolk sac infections hence, more studies are needed to understand the role of this organism in embryonic mortality.
Culture and PCR detection of Campylobacter fetus spp. venerealis from preputial scrapings inoculated into transport and In-Pouch TF media, and saline

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The genus Campylobacter contains multiple species including Campylobacter fetus which is divided in two subspecies: C. fetus ssp. fetus (CFF) and C. fetus ssp. venerealis (CFV). Culture and differentiation of CFF and CFV remain challenging due to the fastidious growth requirements and biochemical inactivity of these organisms. CFV has adapted to the genital tract of bulls and infected animals become asymptomatic carriers; however, infection in cows leads to reproductive failure due to early embryonic death, abortion or infertility. The disease is notifiable by the Office International des Epizooties, because of its negative economic impact; therefore, animals must be certified CFV-free for international trade. Detection of carrier bulls is important for disease control in a herd, thus it is recommended to culture samples [preputial scrapings (PS)] within 4 h of collection, as CFV is very fragile outside the host. Alternatively, PS can be inoculated into transport enrichment medium (TEM) in case of delays during shipment to the lab for testing. Although culture is the gold standard for CFV detection, many diagnostic labs have used this procedure, but with mixed successes.

We evaluated the detection rate of CFV from 291 PS obtained from different cattle operations in Saskatchewan and Alberta with a history of reproductive failure. Samples were inoculated into Landers TEM (LTEM) and In-Pouch TF medium (IPTF) (Biomed Diagnostics), soon after collection. LTEM samples were sub-cultured onto blood and Skirrow agar media within 24 h of collection; cultures were incubated at 35°C under microaerophilic environment and examined for bacterial growth for 5 days. IPTF samples were tested by real time PCR assay with a few modifications (1), upon arrival at the lab (IPTF-0) or after incubation at 35°C for 48h (IPTF-48). Another set of PS (n=31) were inoculated in both IPTF and phosphate buffered saline (PBS) and subjected to PCR as described above except that PBS samples tested upon arrival at the lab (PBS-0) were kept at 4°C and tested again at 48 h after reception (PBS-48), in parallel with corresponding IPTF-48.

Our results indicate that the detection rate of CFV, using LTEM and culture, was very low (0.7%), compared to the detection of the organism from IPTF-0 and IPTF-48 by PCR (7.2% each). PCR detection of CFV from the 31 PS inoculated in both IPTF and PBS was identical (6.4%), at each test period. All PCR-positive results were validated by melting curve analysis and sequencing, using a CFV ATCC strain 19438 as positive control.

Our data suggest that real time PCR assay may be an important tool for the detection of CFV in a routine diagnostic lab setting. Use of IPTF samples can help test simultaneously for both CFV and Tritrichomonas foetus, another venereally transmitted organism. Furthermore, to reduce cost, PBS samples can be considered for CFV detection by PCR.

Reference
Comparison of different sample preparation methods for bovine vaginal mucus for detection of *Campylobacter fetus* subsp. *venerealis* with real-time PCR

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The laboratory diagnosis of bovine genital campylobacteriosis is challenged by the proper identification of *Campylobacter fetus* subspecies. Although culture is still the gold standard, PCR-based methods have been shown to be a good choice for the detection of *Campylobacter fetus* subsp. *venerealis* (Cfv). Successful Cfv detection is dependent on both the PCR assay used and the sample preparation technique. The objective of this study was to compare real-time PCR results, utilizing different sample preparation techniques, versus direct culture of bovine vaginal mucus for detection of Cfv.

Samples from 50 beef heifers, collected weekly from weeks 4–7 of exposure to infected bulls, were cultured, frozen, and subsequently processed for real-time PCR. Initially, real-time PCR results from 50 samples, prepared with 4 variations of a heat lysis protocols, were compared to culture. These were modifications of a previously described method for bovine preputial samples (1). The 2 heat lysis protocols with the fewest dilution steps generated the most positive and least ambiguous test results and were subsequently compared to culture and with results of real-time PCR on samples prepared with a commercial magnetic bead-based extraction kit, using 200 samples. The magnetic bead-based protocol demonstrated higher sensitivity (Sens=48.4%, P=0.019) and lower specificity (Spec=78.9%, P=0.009) than the first heat lysis protocol, which involved an additional dilution step (Sens=29.4%, Spec=88.2%), but had no significant differences with the second heat lysis protocol (Sens=35.0%, P=0.157; Spec=81.1%, P=0.618).

Overall, the sample preparation methods that worked well for bovine preputial samples did not work well for vaginal mucus. This could have been due to the high viscosity of vaginal mucus, which could cause Cfv loss during processing or repeated sample freezing and thawing. **Although heat lysis of vaginal mucus samples is simple, rapid and promising as a diagnostic approach for detection of Cfv with real-time PCR, it still requires further testing on fresh vaginal mucus samples.**

Reference

*Graduate student presenter
Intra-uterine bacterial species and *Escherichia coli* virulence genes could allow predicting the risk of developing postpartum metritis in dairy cows

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After calving, *E. coli* and other bacterial species colonize the uterus of dairy cows and can cause postpartum metritis. The objectives of this study were to determine the bacterial species present in the uterus of dairy cows during the first week postpartum and their association with the occurrence of postpartum metritis in the subsequent weeks, determine the association between *E. coli* virulence genes and the occurrence of postpartum metritis, and assess whether certain bacterial species and some *E. coli* virulence genes could predict the development of postpartum metritis in dairy cows.

Uterine swabs were collected in the first week postpartum on 497 dairy cows and submitted to the laboratory for bacterial culture and identification of *E. coli* virulence genes. The associations between bacterial species and the occurrence of postpartum metritis were quantified by logistic regression.

Bacterial species present in the uterine swabs were: *E. coli* (52%), *Trueperella pyogenes* (30%) and *Fusobacterium necrophorum* (3%). The prevalence of metritis was 13%. This prevalence was higher (24%) in cows positive for *E. coli* and *T. pyogenes* simultaneously than in the cows positive only for *E. coli* (14%) or *T. pyogenes* (7%). Cows positive for *E. coli* had 2.6 times the risk of developing postpartum metritis than cows without *E. coli*. Two *E. coli* virulence genes (*kpsMTII* and *hra1*) were associated with postpartum metritis with an odds ratio of 4.3 each. The presence of *E. coli* in the uterus was used to predict postpartum metritis in 18% of cows, and the presence of the genes *kpsMTII* and *hra1* allowed prediction of postpartum metritis in 36% and 31% of cows respectively.

*Graduate student presenter

Early detection of certain *E. coli* virulence genes in uterine samples could help to predict the risk of developing postpartum metritis. Future studies could focus on developing bacteriological tests easily applicable on farm.
Trends of bacterial antimicrobial resistance associated with cattle affected with bovine respiratory disease complex (BRDC) in Missouri, USA

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Antimicrobial resistance is a well-recognized problem in treating cattle with BRDC with such antimicrobial agents as ampicillin, penicillin, oxytetracycline, tylosin, and sulfadimethoxine. However, data from recent years have demonstrated marked and increasing resistance to newer antimicrobial agents used in the treatment of BRDC. Therefore a retrospective study was conducted to compare the time periods of 1/1/2005 -12/31/2010 and to 1/1/2011-1/1/2015. Lung samples were obtained from deceased cattle at postmortem at the Veterinary Medical Diagnostic Laboratory (VMDL) or samples mailed from veterinarians in the field. In some cases, samples were obtained from BRDC affected cattle via transtracheal washes. In either case the samples came from herds with both marked death losses and a demonstrated lack of response to antimicrobial therapy. Standard methods of culture were utilized to recover offending respiratory bacterial pathogens from the respiratory tract: blood agar (trypticase soy agar with 5% sheep blood) (SBA); MacConkey agar, and chocolate agar. Aerobic and microaerophilic (95% air-5% CO2) conditions at 35°C were used respectively. Mannheimia haemolytica and Pasteurella isolates were identified with conventional methods or with the use of the Sensititre TM AP-80 automated gram-negative identification system. Histophilus somni identification was based on the observation of growth only on chocolate agar in 95% air-5% CO2, gram-negative coccobacillary morphology, and a positive oxidase test.

Over the 10-year period, Mannheimia haemolytica (N=334) was the most commonly isolated bacterial pathogen from cattle, followed by Pasteurella multocida (N=257); Histophilus somni (N=137); Trueperella pyogenes (N=43), and Pasteurella trehalosi (N=17). Antimicrobial susceptibility testing was performed with the Sensititre TM lyophilized micro-broth dilution minimal inhibitory concentration (MIC) test system.

Based on MIC 50-MIC -90 data, shifts of resistance were noted with Mannheimia haemolytica and the following antimicrobial agents: ampicillin, chlortetracycline, danofloxacin, enrofloxacin, florfenicol, spectinomycin, tilmicosin and tulathromycin from 1/1/2005-12/31/2010 and 1/1/2011-1/1/2015.
Field evaluation of an improved Anaplasma antibody cELISA using recombinant MSP5-GST and evaluation of cross-reactivity of Ehrlichia antibodies

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This study was carried out to further evaluate an improved cELISA for Anaplasma antibody detection using field sera collected in various infection stages including long-term carrier status. The cELISA was evaluated for inter- and intra-laboratory repeatability using an interdependency check set prepared by VMRD, diagnostic specificity/sensitivity using a panel composed of diverse field sera prepared by participating laboratories, cut-off for positive and negative qualitative results, and cross-reactive detection of Ehrlichia antibodies.

Inter- and intra-laboratory repeatability of the cELISA results was 100% against 40 sera using duplicates of 10 positives and 10 negatives in testing by operators in 3 independent laboratories. The diagnostic sensitivity against field sera determined by positive results in both PCR and v1 cELISA was 98.2% (100% and 97.4% against US and Canada sera, respectively). The diagnostic specificity against sera determined by negative results in both PCR and v1 cELISA was 100%. A receiver operating characteristic curve analysis shows that a cutoff of 30% inhibition optimizes the assay for maximum specificity while providing excellent sensitivity. Furthermore, detection of cross-reactive Ehrlichia antibodies reported in the v1 cELISA made with recombinant MSP5-MBP was not observed in the cELISA improved with recombinant MSP5-GST. This discrepancy between current results and a previous report (1) could be due to non-specific binding to the MBP fusion protein used in the v1 cELISA.

In summary, the data in this field evaluation together with previously published data (2) strongly support that the improved cELISA is an effective high resolution Anaplasma antibody detection tool suitable for screening of animals with acute and carrier infections.

References
016 Defining the long-term duration of parasitemia and antibody response in cattle infected with *Babesia bovis* and evaluating serodiagnostic tools

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Defining 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. In this study, parasitemia and *B. bovis*-specific antibody response were defined in calves infected with high and low doses of 3 *B. bovis* strains including 2 attenuated and 1 pathogenic. All 6 experimentally infected cattle had recurrent parasitemia lasting >10 months post-infection, even those infected with a low dose of attenuated *B. bovis* strains, indicating persistent infection capacity of all 3 *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 the 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 6 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 at days with no detectable parasitemia, indicates the relative advantage of antibody-based testing 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 when parasitemia was still detectable by PCR. Moreover, the initial positive detections by the cELISA in low-dose challenged-animals were approximately 6 days delayed compared to IFA. 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 serodiagnostic assay with better diagnostic sensitivity/specificity (>98%) against sera from global bovine herds and its use may be pivotal in preventing the spread of *Babesia bovis* from endemic to non-endemic areas.

Reference
Swine influenza virus (SIV) is a highly contagious viral infection of pigs, resulting in significant economic losses in the swine industry and posing a significant threat to human health through zoonotic transmission. SIV subtypes are defined by the surface glycoproteins: hemagglutinin and neuraminidase, with H1N1, H3N2, and H1N2 representing the predominant subtypes in swine.

We have validated an SIV testing workflow consisting of high-throughput nucleic acid purification, SIV detection, and SIV subtyping from porcine nasal swab samples. SIV can be detected using the USDA-licensed VetMAX™-Gold SIV Detection Kit, a single-tube one-step real-time RT-PCR kit for the rapid and accurate screening for influenza A. The assay targets 3 independent regions of the SIV genome to dramatically limit the number of false-negatives due to mutation of the viral genome. The detection assay also incorporates multiple degenerate primers and probes designed to detect all known strains of SIV. It is multiplexed with an internal positive control (IPC) to monitor nucleic acid recovery and PCR inhibition. Laboratories wishing to obtain more information about SIV-positive samples can utilize the VetMAX™-Gold SIV Subtyping Kit to further characterize their samples and confirm positive results. The subtyping kit is a pair of single-well real-time RT-PCR assays to detect and differentiate the H1, H3, N1 and N2 alleles.

We validated the screening and workflow by testing >100 SIV-positive and >100 SIV-negative porcine nasal swab field samples and virus isolates originating from diverse geographic regions in the US with the screening and subtyping kits. The SIV status and subtype of each sample was confirmed prior to the start of the study with virus isolation (VI) and/or whole genome sequencing. Collaborator laboratories purified viral nucleic acid using the MagMAX™-96 Viral RNA Isolation Kit (AM1836) and MagMAX™ Express-96 Magnetic Particle Processor. Extracted nucleic acid (8uL) was tested with the VetMAX™-Gold SIV Detection Kit and VetMAX™-Gold SIV Subtyping Kit on the AB 7500-Fast Real-Time PCR System.

Results of validation testing were used to determine diagnostic sensitivity and specificity for each kit. Detection with the VetMAX™-Gold SIV Detection Kit resulted in calculated diagnostic sensitivity and specificity values of 98.4% and 99.1%, respectively. The VetMAX™-Gold SIV Subtyping Kit produced >97% sensitivity and specificity for identifying the SIV subtype from nasal swab samples. **This study indicates that RNA isolated from diagnostic porcine nasal swab samples, tested with the VetMAX™-Gold SIV Subtyping Kit in conjunction with the VetMAX™-Gold SIV Detection Kit, provides an economical and rapid solution for SIV screening and subtype identification.**
Porcine respiratory disease complex (PRDC) is one of the most economically significant diseases of growing pigs worldwide. The most common pathogens that play a role in PRDC are: *Porcine reproductive and respiratory disease syndrome virus* (PRRSV), *Influenza A* (FluA) virus, *Porcine circovirus 2* (PCV2), and *Mycoplasma hyopneumoniae* (Mhyo). Even though PRDC is worldwide, very little information is published about the prevalence of these pathogens in Western Canada. Therefore, the purpose of this study is to assess the value of using oral fluids compared to the already ongoing on-farm routine disease surveillance being performed using the “gold-standard” samples of nasal swabs or blood for each of the respiratory pathogens.

We collected 6 oral fluids (OF) samples per month from each of 10 grower-finisher farms for 5 consecutive months over the winters of 2013-2014 and 2014-2015 (600 samples total). Real-time PCR was used to detect viral nucleic acid in all OF samples. Barns were considered positive for a pathogen if at least one pen tested positive for that pathogen. Preliminary results on 258 OF samples have been completed for FluA, PRRSV, and PCV-2 (year 1). Overall, 9 (3.5%) samples were positive for FluA, 47 (18.2%) for PRRSV, and 156 (60.5%) for PCV2. The barn-level prevalence of FluA, PRRSV, and PCV2 were 30%, 40%, and 80% respectively. Furthermore, more than one pathogen was found in 6 farms, and all 3 pathogens (FluA, PRRSV, and PCV2) were found in one farm. Nasal swabs (NSSW) received with some of the oral fluid samples were sequenced and interestingly the positive oral fluids were correlated to positive NSSW as a cluster IV H3N2 in one farm and pandemic H1N1 in another farm.

**OF are convenient, reliable and economic pooled diagnostic samples that are easy to collect with minimal training, which makes them ideal for on farm routine disease surveillance.** The complete results of the 2-year study will be presented at the conference.

**References**


Detection of type 2 PRRS virus in the fetal implantation sites of pregnant gilts

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The pathogenesis of fetal death during reproductive porcine reproductive and respiratory syndrome (PRRS) is still poorly understood. Although it is believed that PRRSV infection of the fetus is transplacental in nature, exactly how the virus crosses the pig's diffuse epitheliochorial placenta remains unclear. The objective of this study was to clarify the routes of PRRSV transmission in the fetal implantation sites by identifying and quantifying PRRSV-infected cells within the uterus and fully attached fetal placenta using immunohistochemistry.

On gestation day, 85±1, 113 PRRS virus-naïve pregnant gilts were inoculated with PRRSV (105 TCID₅₀ total dose) and 19 negative control gilts were sham inoculated. At 21 days post inoculation, dams and their litters were euthanized for postmortem examination. Samples of uterine/placental tissue adjacent to the umbilical stump of each fetus were analyzed by an in-house qPCR to quantify PRRS viral load. The corresponding paraffin-embedded uterine/fetal placenta sections were subjected to immunohistochemistry using mouse monoclonal antibodies against PRRSV SDOW17 antigen, antibody detection with DAKO EnVision+System and AEC (3-amino-9-ethylcarbazole) as a chromogen. The number of PRRSV infected cells per 1 mm² of endometrium and the fetal placenta was determined by Image ProPlus software.

PRRSV immunopositive cells were detected in all samples of uterus and the fetal placenta from PRRSV-infected pregnant gilts. The largest numbers of PRRSV-immunopositive cells were found at the endometrial placental junction, and they were composed of primarily tissue macrophages and fewer uterine epithelial cells. Fewer PRRSV infected macrophages were observed in the fetal placenta in the proximity of the maternal-fetal interface. In the endometrium, PRRSV-immunopositive macrophages were rare and located away from blood vessels in the lamina propria. Epithelial cells of the uterine glands occasionally demonstrated strong PRRSV-immunopositive staining. The numbers of PRRS-immunopositive cells in the endometrium and in the fetal placenta determined by immunohistochemistry were both positively related to PRRS viral load analyzed by qTR-PCR (β=0.462, 2.556, respectively; P<0.001; linear mixed model) indicating a significant association between 2 diagnostic techniques.

Detection of PRRSV antigen in the macrophage-like cells present at the maternal-fetal interface and in the fetal placenta suggests a cell-associated route of virus transmission by migration of infected cells from the endometrium to the fetal-placental membranes.

Funding for this project was provided by Genome Canada, Genome Prairie, and BIVI PRRS Research Award. We acknowledge Jan Shivers from the University of Minnesota, Melissa Koehnlein, and Dr. Dale Godson from Prairie Diagnostic Services for their technical assistance in immunohistochemistry.

*Graduate student presenter
Assessment of the antibody response in pigs directed against Porcine circovirus 2

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Porcine circovirus 2 (PCV-2) is the causative agent of the post-weaning multi-systemic wasting syndrome (PMWS), a disease that is associated with high mortality rates in pigs. Vaccination has decreased prevalence of PMWS in farmed animals; however, the antibody levels that reliably determine protection from PCV-2 infection and/or disease are still unknown. Four different serologic assays are currently used to assess the antibody response against PCV-2: a) an ELISA based on a truncated PCV-2-capsid protein (aa43-233 of PCV-2-capsid), which likely detects antibodies directed against linear epitopes; b) an ELISA using a small peptide (aa169-180 of PCV-2-capsid) that represents a linear epitope; c) an immunofluorescent assay (IFA) employing PCV-2-infected and fixed tissue cells; and d) a virus-neutralizing assay (VNA) assessing the PCV-2-neutralizing activity of pig sera.

We optimized existing serologic tests for the detection of PCV-2 antibodies and compared the results from these assays using a panel of 80 serum samples belonging to nursery and fattening pigs of 11 different farms in Alberta. We transformed the conventional VNA into a high-throughput format resulting in higher accuracy. Furthermore, we developed a PCV-2-particle ELISA, which exposes only conformational but not linear epitopes. We replaced the conventional capsid ELISA with a full-length capsid-based Western blot to warrant full denaturation (linearization) of all epitopes, and we performed the aa169-180 peptide ELISA as described previously (1). The screening of swine sera showed that 96.2% were positive in PCV-2-particle ELISA and 92.5% in VNA; a significant positive correlation was found between these two assays (Spearman’s coefficient $r^2=0.33; p<0.05$). Furthermore, 89.7% of swine sera reacted to the recombinant PCV2-capsid in Western blot and 95.0% reacted to the peptide ELISA; a positive significant correlation was observed between Western blot and the peptide ELISA results (Spearman’s coefficient $r^2=0.29; p<0.001$).

This study demonstrates the presence of antibodies against both conformational and linear epitopes of PCV-2 in the serum of naturally-infected animals employing selective and sensitive assays. Future work will reveal whether serologic assays can be used to determine the status of protection and health or disease in PCV2 exposed and/or vaccinated pigs.

Reference

*Graduate student presenter
Measuring by quantitative PCR the critical number of *Lawsonia intracellularis* that cause production losses on a commercial pig farm

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*Lawsonia intracellularis* is the causative agent of porcine proliferative enteropathy (PE), resulting in diarrhea, reduced weight gains, and increased feed to gain ratios, which all impact on the cost and efficiency of production. The prevalence of infection within herds can be very high (> 80%), but only a small proportion of pigs show clinical signs. Although the majority of pigs may be subclinically affected, production losses are still recorded.

Quantitative PCR assays (qPCR) can calculate *L. intracellularis* numbers in feces in real time, and previous studies have demonstrated that both diarrhea and reduced average daily gain are correlated with increased numbers of *L. intracellularis* (Collins et al., 2014). Experimental challenge trials indicate that large reductions in average daily gain (ADG) occur once pigs excrete more than $10^7$ *L. intracellularis* per gram of feces (131 g/day), but smaller reductions still occur when more than $10^6$ *L. intracellularis* are excreted (Collins et al., 2014). Environmental factors along with the pig’s age, diet, genetics, immune response, and intestinal health will all impact on the clinical response to infection, so the critical number of *L. intracellularis* that cause production losses needs to be calculated under commercial conditions.

Using pooled pen fecal samples and pen weights, this study aimed to identify the critical threshold of *L. intracellularis* in commercial pig herds. Grower pigs (n = 530) were randomly allocated into 24 pens and pooled fecal samples were collected from the pen floor every 2 weeks from 10 to 18 weeks of age. Average daily gain and feed intake per pen were recorded at 10, 12, 15 and 18 weeks age and correlated against *L. intracellularis* numbers over the same periods.

A negative correlation between Log$_{10}$ *L. intracellularis* numbers and ADG was demonstrated ($r = -0.305$, $p= 0.146$), indicating that as bacterial numbers increased, weight gains decreased. Exponential regressions were fitted to the data between 10 and 15 weeks of age, corresponding to the peak in infection. The regression predicted that ADG was reduced from 847 g/day to 707 g/day when fecal excretion of *L. intracellularis* increased from $10^7$ to $10^8$ *L. intracellularis*, a difference of 140 g/day or a total of 2.9 kg over 21 days ($R^2 = 0.43$). ADG was only reduced minimally over 21 days (322g) with a 10-fold increase from $10^6$ to $10^7$ *L. intracellularis*. The qPCR proved a useful tool for diagnosing PE, quantifying the critical pathogen load, and evaluating strategies to control disease in commercial herds.

Financial support was provided by the Australian Pork CRC for High Integrity Pork.  
*Reference*  
Next-generation sequencing and quantitative PCR can identify the impact of antimicrobials on the abundance of bacteria in pig feces

Alison Collins, Bethany Bowring, Sasha Jenkins

Dietary changes, stress, the loss of maternal antibodies, and environmental changes make pigs particularly vulnerable to intestinal pathogens following weaning (1). Commensal bacteria in the intestine, such as lactobacilli, help prevent pathogen colonization by competitive exclusion and excretion of bacteriocins. Diarrhea caused by enterotoxigenic \textit{E. coli} (ETEC) is a significant problem in post-weaned pigs, leading to dehydration, toxemia, reduced growth rates, and mortalities.

Antimicrobials are used to prevent and treat post-weaning diarrhea, but are not always successful, either due to resistance by the pathogens or due to disturbance in the balance of commensal and pathogenic bacteria in the intestine. In this study, fecal samples were collected from weaner pigs with and without diarrhea from 2 non-medicated and 4 medicated piggeries to identify the impact of antimicrobials on commensal and pathogenic bacteria.

Quantitative polymerase chain reaction (qPCR) was used to determine the actual numbers of lactobacilli, total \textit{E. coli}, \textit{Enterobacteriaceae}, fimbrial type 4 (F4) \textit{E. coli} (ETEC), and total eubacteria per gram of dry weight feces. Standards for the qPCR were developed from pure cultures of reference bacteria. Differences in the relative abundance of bacterial groups were evaluated by next-generation sequencing (NGS) of PCR amplicons using the universal 16S rRNA primers to regions V4/5. All data were analyzed using the QIIME pipeline with appropriate quality controls. Chimeras were identified and removed before assigning operational taxonomic units. The relationship between antimicrobial use and microbial abundance was assessed using general linear model (GLM) and canonical correspondence analysis (CCA).

Medicated pigs had reduced numbers of protective lactobacilli and total eubacteria combined with increased numbers of ETEC, total \textit{E. coli}, and \textit{Enterobacteriaceae}, demonstrated by both NGS and qPCR. Not only were the antimicrobials ineffective against ETEC, but they reduced the number of protective lactobacilli, potentially leading to an exacerbation of diarrhea. NGS also identified a higher abundance of some butyrate-producing bacteria in medicated pigs. However, other lactate and butyrate producers decreased in abundance in response to medication. \textbf{Lactate and butyrate producers are known to be important in maintaining intestinal health, so the development of qPCR assays for these bacterial groups may prove useful in the future to measure intestinal health in weaner pigs.}

Financial support was provided by the Australian Pork CRC for High Integrity Pork.

Reference
023 Best practices to detect PEDV, TGEV, and PDCoV

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We have developed a multiplex real-time polymerase chain reaction assay for detection and differentiation of 3 swine enteric coronaviruses, Porcine epidemic diarrhea virus (PEDV), Porcine deltacoronavirus (PDCoV), and Transmissible gastroenteritis virus (TGEV). The clinical signs of all 3 enteric viral infections are very similar, making exact diagnosis a challenge in the absence of laboratory tests. The PEDV variant affecting US swine was first reported in April 2013. This US variant is particularly virulent and leads to severe diarrhea in swine. In February of 2014, a new swine coronavirus with clinical characteristics similar to PEDV and TGEV was identified in the state of Ohio. According to clinical reports, the new coronavirus, Porcine deltacoronavirus (PDCoV), exhibits a lower mortality rate than PED.

In response to the increase in number of PED cases and the concern that PED and TGE share the similar clinical signs, we initially developed and commercialized a real-time RT-PCR multiplex assay for the detection and differentiation of PEDV and TGEV viral RNA (EZ-PED/TGE MPX). However, soon after the identification of the PDCoV virus, we re-optimized the assay to include primer and probe sets for the specific detection and differentiation of PDCoV from PEDV and TGEV without affecting specificity and sensitivity.

By the end of July 2014, after extensive validation of this new multiplex assay by collaborators and different diagnostic labs throughout the US, Tetracore released their new multiplex assay, EZ-PED/TGE/PDCoV MPX real-time RT-PCR reagents. The assay provides specific detection and differentiation of the PEDV, TGEV, and PDCoV viral RNA, with optional addition of inhibition control(IC), in a single reaction. This multiplex assay was designed to amplify 2 highly conserved regions of each the PEDV, TGEV, and PDCoV viral RNA, thus providing broad detection of each virus by ensuring detection despite mismatch or mutation in one of the target regions. The assay is able to differentiate each viral RNA target by using different reporter dyes. Two formats of the multiplex assay have been optimized and validated in order to facilitate the analysis of results and minimize cross-talk between channels in various real-time PCR instruments: one format for use on the ABI 7500 instruments, and the other for use on all other real-time PCR instruments.

In this presentation we describe the results of testing EZ-PED/TGE/PDCoV MPX assay with several types of swine samples including oral fluids and fecal samples. Results from validation of the assay for use on both lab based instrument and Tetracore’s portable real-time PCR instrument T-COR 8™ are described.
024 Real-time PCR reagents for the detection and differentiation of swine enteric coronaviruses

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Porcine epidemic diarrhea virus (PEDV) and Porcine deltacoronavirus (PDCoV) represent new threats to the swine industry. To aid in early detection of virus, monitor shedding, or differentiate viral species, PCR has been a useful diagnostic tool. IDEXX has developed real-time PCR reagents to detect and differentiate viral RNA from PEDV and PDCoV in a multiplex reaction. Additionally, reagents for detection of RNA from PEDV, PDCoV, and TGEV have been developed. All assays use an internal control approach based on detection of endogenous swine RNA, referred to as the internal sample control (ISC).

The PCR reaction mix is prepared by mixing equal parts of RealPCR™ RNA Master Mix and target-specific detection mix for a total volume of 20 μL x number of samples to be tested. With the sample volume being 5 μL per reaction, the total volume for one PCR reaction is 25 μL. The real-time PCR program consists of one cycle at 50°C for 15 minutes and 95°C for 1 minute, followed by 45 cycles of 95°C for 15 seconds, and 60°C for 30 seconds.

In this performance study for the RealPCR PEDV/PDCoV Multiplex assay, samples consisted of either synthetic oligonucleotides or nucleic acid purified from clinical samples. Clinical samples (fecal swabs and oral fluids) were purified using a commercial total nucleic acid extraction kit. The efficiencies and correlation coefficients for each design were determined using serial dilutions of synthetic DNA. All target designs maintained efficiencies of 95%–105% with R² values of ≥0.994 and detected at least 10 copies per reaction. To ensure no interference and/or competition between target and ISC reactions, multiplexed sensitivity testing was performed for all designs. Copies of the target sequence (PEDV or PDCoV) were amplified in the presence or absence of artificially high concentrations of ISC. High levels of ISC had no impact on the detection of 10 copies of either PEDV or PDCoV. To confirm that the ISC design detects swine RNA and not genomic DNA, the reverse transcriptase (RT) contained in the RealPCR RNA Master Mix was inactivated before addition of sample. Inactivation of RT resulted in complete loss of ISC signal. Sensitivity and specificity for PEDV and PDCoV were evaluated using purified total nucleic acid from samples of known status. The PEDV/PDCoV multiplex had PEDV sensitivity of 99.5% (n=191) and PDCoV sensitivity of 100% (n=44). Both designs had 100% specificity.

These results demonstrate the high sensitivity and specificity of the RealPCR swine enteric coronavirus reagents. The reagents are configured as either single target, with the detection of RNA from PEDV, PDCoV or TGEV, or as a PEDV/PDCoV multiplex. All configurations include an ISC for the detection of swine RNA as an internal control.
Porcine epidemic diarrhea (PED) is an infectious disease that attacks breeding-age as well as suckling neonatal pigs, with an 80-100% morbidity and mortality rate among piglets. Detection of the PED virus (PEDV) can be difficult, and there is no easy treatment. An outbreak that emerged in the USA in 2013 has killed an estimated 7 million swine so far, reducing pork production by about 10%. While PEDV does not cause a food safety concern for humans, U.S. consumers have faced higher prices of pork due to the impact of PEDV on supply. The U.S. epidemic has been very costly to hog farmers through lost production, added costs of biosecurity, and vaccination. PEDV is spreading fast through Latin America. The World Organization for Animal Health (OIE) reported outbreaks among others in Mexico, Dominican Republic, Columbia, and Peru.

A critical step in the fight against PEDV is the reliable detection of the virus. Several technologies based on ELISA and PCR principles are commercially available. Determination of the presence of the virus can be done fastest with PCR. PEDV induces signs very similar to the TGEV (transmissible gastroenteritis virus); therefore the need to differentiate both viruses.

In collaboration with the University of Minnesota, QIAGEN developed the virotype PEDV/TGEV RT-PCR Reagent to detect PEDV RNA and differentiate PEDV infections from TGEV infections; all in a single reaction tube, combining the analysis of RNAs from each virus and an internal control.

The present study investigated the performance of the virotype PEDV/TGEV RT-PCR Reagent for the identification of PEDV and its differentiation from TGEV. Analytical and diagnostic sensitivity and specificity as well as reproducibility were assessed for different sample types: fecal, oral fluids (saliva), environmental, and feed samples. The assay was further validated by analyzing samples from different regions: Europe, North America, Latin America, Asia.

The virotype PEDV/TGEV RT-PCR Reagent enables reliable detection of PEDV and differentiation from TGEV.
026 Rapid response to African swine fever: development and validation of QIAGEN's new virotype ASFV PCR Kit

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African swine fever (ASF) is one of the most severe viral diseases affecting swine production. It is a highly contagious viral disease of domestic and wild pigs of all ages leading to subclinical or highly lethal infections. The causative agent, African swine fever virus (ASFV), is a complex DNA virus and is the only member of the Asfarviridae family.

An increasing number of cases of ASF have been detected in wild boar and domestic pigs in Eastern European countries. The risk of ASFV spreading to other countries through contaminated meat, animals, or vehicles is very high. EU Member States are screening for ASFV and also Classical swine fever virus (CSFV), whose signs are very similar. With surveillance and prevention being stepped up, there is a need for a user-friendly, reliable diagnostic solution for both diseases.

QIAGEN provides diagnostic solutions for both pathogens from sample extraction to detection. The new ready-to-use virotype ASFV PCR Kit (FLI-B 670) is a duplex real-time PCR for early detection of ASFV DNA from porcine serum, plasma, EDTA blood, tissue, and swab samples. The Kit contains a Master Mix, a positive and a negative control. An internal control (porcine $\beta$-actin) is included to monitor extraction and amplification, and to exclude the possibility of false negatives results. The high sensitivity of virotype ASFV PCR allows identification of the pathogen in individual as well as in pooled samples within 2 hours. Furthermore the virotype ASFV PCR Kit can be used alongside the virotype CSFV RT-PCR Kit (FLI-B 517) for the detection of CSFV.

As recommended by OIE and FLI, DNA and RNA from ASFV and CSFV respectively can be extracted simultaneously using the QIAamp Viral RNA Mini Kit (QIAGEN). For automated sample preparation, QIAcube can be used. The extracted nucleic acids can then be tested in the same cycle run for both ASFV and CSFV using the virotype ASFV PCR Kit and virotype CSFV RT-PCR Kit.

Validation was conducted on ASFV-positive pig and wild boar samples experimentally infected with the Armenia, Sardinia, and Kenia05 strains. DNA was extracted from blood, spleen, lymph nodes, tonsils, salivary gland, oral-pharyngeal swabs, and bone marrow. When testing positive samples, the assay demonstrated high sensitivity. Analytical sensitivity was determined by a titration series of in-vitro ASFV DNA. The detection limit of the kit was shown to be 10 copies of ASFV DNA.

By testing 165 ASFV-negative samples, a specificity of 100% was determined. In addition, pig samples positive for other porcine viral pathogens were tested - no cross-reactivity was detected. The virotype ASFV PCR Kit is a highly sensitive and specific solution for the reliable and rapid detection of ASFV-DNA in samples from pigs and wild boar. In combination with the QIAamp Viral RNA Mini Kit and the virotype CSFV RT-PCR Kit, QIAGEN provides a complete workflow for ASFV and CSFV detection, saving precious lab time.
027 Development and validation of a new ASFV real-time PCR

Elodie Coulon, Sandrine Moine, Immanuel Leifer
(Thermo Fisher Scientific, Lissieu, France)

African swine fever virus (ASFV) causes a notifiable, highly contagious disease that can cause enormous economic losses. The disease is widely endemic in many parts of Africa, in parts of southern Europe, and is increasingly becoming a threat in eastern Europe. As there is still no vaccine or treatment available, monitoring and controlling of the disease is of utmost importance. In order to improve diagnostics, Thermo Fisher Scientific has developed and validated a new duplex real-time PCR kit.

For the development of a reliable, sensitive, and specific rtPCR system, >450 different ASFV sequences representing the p72 protein encoding region were aligned. The new assay is composed of a duplex rtPCR including an internal control to ensure reliable DNA extraction. For extraction of viral DNA from field samples, the MagMax Pathogen RNA/DNA Kit and the MagVet Universal Isolation Kit were validated. In order to demonstrate the sensitivity and specificity of the new LSI VetMAX™ ASFV kit, different internal and field studies including animal infection experiments (INIA, Valdeolmos, Spain; CVI, Netherlands; Germany) were carried out. In total, about 1,600 negative samples from ASFV-free regions (Germany, Spain) and additionally 33 different pathogens were tested to demonstrate specificity of the assay. For validation of the sensitivity, ~100 ASFV-positive samples from Africa and Europe were tested. The limit of detection (LDPCR) was determined by serial dilution of a plasmid carrying a specific ASF sequence (pASF). The efficiency of the PCR reaction was identified by using dilutions of \(10^{-4}\) to \(10^{-11}\) of the pASF plasmid.

Test results of the ASFV-positive samples showed 100% sensitivity in all tested sample materials (blood, serum, and tissue samples). A serial dilution of the ASFV target sequence led to a limit of detection (LOD) of 16 genome copies per PCR reaction. The experimental LOD was \(5 \times 10^3\) copies per mL in serum and \(1 \times 10^4\) copies per mL in blood. By testing 1,600 negative samples, a specificity of 100% was demonstrated. Additionally, all 33 alternatively infected samples scored negative in the ASFV-specific assay.

In conclusion, the LSI VetMAX™ ASFV kit fulfils all the validation criteria of PCR characteristics and a complete method required by the U 47-600-2 standard.
Detection of astrovirus in 50-year-old archived tissue samples of cattle with nonsuppurative encephalitis

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Since the 1960s, the neuropathological diagnostic services of our division have recorded cases of sporadically occurring non-suppurative encephalitis in about 15% of adult cattle with neurological diseases (1,2). Clinically, affected animals show abnormal behavior, sensitivity and locomotion, and subacute to chronic course of disease. Neuropathology is characterized by perivascular cuffs, gliosis and neuronal necrosis, mainly involving the rhombencephalon, mesencephalon, and diencephalon, and accompanied by mononuclear meningitis. This inflammatory pattern suggests a viral etiology, but in most cases the causative pathogen remained unknown. Using a next-generation sequencing approach and targeted molecular testing, we described a novel bovine astrovirus (BoAstV-CH13) in a series of recent cases of sporadic nonsuppurative encephalitis (3).

In the present study, we addressed the question of whether BoAstV is detectable in historical samples that had been included in the first reports of the disease. We selected formalin-fixed and paraffin-embedded tissue blocks of the caudal brainstem, the cerebellum, and the cerebrum of 14 cases of histologically confirmed cases of sporadic nonsuppurative encephalitis and 6 negative control animals from our archives. All had been diagnosed between 1958 and 1976. Tissues were re-embedded in paraffin and analyzed by in-situ hybridisation for the presence of BoAstV-CH13 RNA using 2 distinct digoxigenin-labelled RNA probes (A and B). Twelve out of 14 diseased animals (86 %) revealed a clear neuronal RNA labelling with probe A in at least one of the brain areas examined. Labelling was absent in the tissues of negative control animals. Strikingly, probe B failed to detect the viral RNA in the historical cases, but gave a clear signal on positive control sections derived from more recent cases. This failure may be related to long-term tissue storage, but could also indicate the involvement of astroviruses genetically diverse from BoAstV-CH13. Taken together, these results support the notion that astroviruses have been present in the bovine population for decades causing encephalitis, but have gone undetected.

References
1. Fankhauser R. Sporadic meningoencephalomyelitis in cattle [article in German]. Schweiz Arch Tierheilk 1961;103:225-235.

*Graduate student presenter
Rabies in African wild-dogs (*Lycaon pictus*) in northwest South Africa linked to a dog (canid) rabies cycle

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Rabies, is a fatal viral zoonotic disease, and one of the most feared in medical history. In Asia and Africa (including South Africa), the disease is a significant public health concern, given that an estimated 55,000 and probably up to 70,000 human deaths occur annually mainly in the developing countries. In South Africa, the rabies situation is complex due to the existence of domestic and wildlife rabies cycles and cross-species transmission of the virus.

On December 26, 2014, a male African wild-dog, owned by the Madikwe Nature Reserve, in the northwest of the country bordering Botswana, apparently entered a swimming pool and bit 3 people before it was shot. The carcass was submitted for rabies diagnosis. On January 27, 2015, a carcass of a female wild-dog from the same nature reserve was also submitted for rabies diagnosis. Smears were prepared from a composite of brain parts of the partly decomposed central nervous system tissue (including brainstem and hippocampus), acetone-fixed and stained with a fluorescein isothiocyanate (FITC)-labelled polyclonal lyssavirus conjugate. The rabies-positive samples were antigenically characterized using a panel of 16 anti-nucleoprotein (anti-N) monoclonal antibodies for differentiation of southern African lyssaviruses. Subsequently, high quality total viral RNA was Trizol-extracted and subjected to next-generation sequencing technology. The generated nucleotide sequences were subjected to phylogenetic analyses with other previously characterized dog and jackal virus sequences using computer algorithms.

Typical apple-green fluorescing viral aggregates were observed in all areas of the smears from the 2 wild-dogs. The reactivity patterns obtained on the panel of Mabs for both viruses was consistent with that of southern African dog (canid) viruses that are maintained in dogs, jackal species, and the bat-eared fox. This observation probably demonstrates a very close link and cross-species transmission of the RABV infection between domestic and wildlife hosts in this part of the country. These data further underscore the complexity of rabies epidemiological cycles in South Africa and the challenges posed for the elimination of the disease from both domestic and wildlife reservoirs and vector species. Despite the immunization of domestic dogs, it appears that rabies epizootics are sustained in the northwest of South Africa by jackals and any other wildlife species that will subsequently act as source of infection for domestic dogs, thereby posing a serious public health threat.

References
Extended-spectrum β-lactamase-producing *E. coli* isolated from wild birds in Saskatoon, Canada, 2014

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*Escherichia coli* is a gram negative, ubiquitous colonizer and opportunistic pathogen of humans and animals. Resistance to the third-generation cephalosporins (e.g., ceftriaxone, ceftiofur) conferred by the extended-spectrum β-lactamases, particularly the CTX-M type enzymes, are increasingly encountered, limiting the therapeutic options available to clinicians. Because *E. coli* (including the human pandemic strain ST131) readily transmits between animals and humans, a complete appreciation of the epidemiology of resistance requires consideration of animal reservoirs. In Canada, no investigations targeting *E. coli* in wild birds have been published. A 2014 report describing CTX-M positive *E. coli* in gulls in Alaska was the first of its kind in North America.

In the present study, we characterized the antimicrobial susceptibility of *E. coli* isolated from wild birds which were presented to the WCVM wildlife and exotics service. Prior to the initiation of treatment, cloacal swabs were collected from 75 wild birds including 21 great horned owls, 25 crows, and 29 American robins. CHROMagar ESBL and MacConkey agars were used for the selective isolation of *E. coli*. Antimicrobial minimum inhibitory concentrations were determined by broth microdilution using the Sensititre system; ampicillin, amoxicillin + clavulanate, cefoxitin, ceftiofur, ceftriaxone, tetracycline, trimethoprim + sulfamethoxazole, sulfisoxizole, chloramphenicol, nalidixic acid, ciprofloxacin, and gentamicin were included. Isolates resistant to ceftiofur or ceftriaxone were screened for CTX-M type ESBLs by PCR.

Overall, *E. coli* were isolated from 51 (68%) of birds; recovery rates varied from 44.8% for American robins to 92.0% for crows. Colonization with pan-susceptible organisms was the most commonly identified phenotype, occurring in 45 (88.2%) of colonized birds. Among resistant *E. coli*, ampicillin resistance was most common including 17 isolates from 6 birds. Nine multidrug-resistant isolates (resistant to ≥3 drug classes) from 3 birds were found. Two birds were colonized with CTX-M producing *E. coli*: a robin with isolates resistant to ampicillin, cefoxitin, ceftriaxone, ceftiofur, tetracycline, sulfisoxizole, trimethoprim + sulfamethoxazole, nalidixic acid, and gentamicin, and a crow with isolates resistant to ampicillin, ceftiofur, ceftriaxone, nalidixic acid, and ciprofloxacin.

The global emergence CTX-M producing *E. coli* is a serious threat to the ability of clinicians to treat commonly occurring infections such as community acquired UTIs. These findings demonstrate that antimicrobial resistance extends beyond humans where it is most widely recognized, and into wildlife. Additional studies are required to determine the role that animals play in the epidemiology of antimicrobial-resistant *E. coli*. 
In recent years, viruses that cause no overt disease in bats have spilled over to humans and other species causing serious and often fatal disease (1). These include the coronaviruses that cause SARS (severe acute respiratory syndrome), MERS (Middle-East respiratory syndrome), and PED (porcine epidemic diarrhea). Our objective is to explore the hypothesis that bat innate immune responses stringently regulate bat-virus interactions leading to non-pathogenic persistent viral infections and that physiological and immunological stressors disrupt this balanced relationship increasing virus replication and, consequently, the possibility of spillovers.

We have recently shown that little brown bats (Myotis lucifugus) and big brown bats (Epitesicus fuscus), the predominant hibernating species in western Canada, are persistently infected with closely related coronaviruses. Co-infection of hibernating M. lucifugus with the fungus Pseudogymnoascus destructans, which causes the deadly white nose syndrome, increases systemic cytokine expression (2) and also increases coronavirus gene expression by over 60-fold. These data and access to primary E. fuscus cells allows us to study bat-virus interactions between a coronavirus and its natural host.

Here we present data comparing the response of human and bat cells to synthetic inducers of innate anti-viral responses. We designed primers that would specifically detect and quantitate transcripts for selected bat and human antiviral cytokines. Synthetic TLR ligands such as polyI:C, ssRNA40, and CpG oligodeoxynucleotides were used to stimulate human fibroblasts (MRC-5) and primary E. fuscus kidney cells. The transcripts for several interferon and interferon response genes were then measured using quantitative real-time PCR. **We have identified significant differences in the innate response between human and bat cells in terms of interferon-β and interferon stimulated gene expression.** The differences are interesting as they might explain why bats are able to persistently harbor these viruses. **Our results are the first attempt to compare innate responses in humans and bats and will form the basis of our investigations to determine why viruses that are innocuous to bats behave very differently in humans.**

**References**


*Graduate student presenter
As skin disease in horses is common and varied, finding efficient and effective diagnostic methods is useful for both clinicians and clients. Fine needle aspiration (FNA) is commonly used to diagnose skin disease in smaller companion animals such as dogs and cats, but its use in horses appears to be less frequent. To determine why this may be the case, equine veterinarians in Western Canada were surveyed to determine their opinions about FNA and a retrospective study of 15 years of diagnostic submissions was performed to compare the perceived to actual value of FNA in the diagnosis of skin disease in horses.

Equine practitioners surveyed viewed FNA as a quick, easy, economical and minimally invasive technique that could be valuable in diagnosing disease and aid in treatment planning. However, most of the surveyed veterinarians indicated that they rarely chose to use FNA in the diagnosis of skin disease due to a perception that the sample quality and diagnostic yield of the technique was poor and that there was a very narrow range of diseases the technique was useful for diagnosing.

Analysis of the FNA cytology samples submitted to a Western Canadian veterinary diagnostic laboratory over a 15-year period showed that a wide variety of equine skin disease conditions were diagnosed using FNA. However, as per practitioners’ perceptions, the frequency of non-diagnostic results was significantly higher in equine FNA submissions than for those in dogs and cats, but this was also true for equine skin biopsies. Therefore, although biopsy is perceived by Western Canadian equine veterinarians to be more diagnostically useful than cytology, based on analysis of diagnostic submissions over the past 15 years, both equine skin biopsy and cytology submissions are more frequently non-diagnostic than canine and feline skin submissions.

*Graduate student presenter
Serology and molecular diagnostics of Epizootic hemorrhagic disease virus (EHDV)

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Epizootic hemorrhagic disease virus (EHDV) is a member of the genus Orbivirus in the family Reoviridae, and is closely related to Bluetongue virus (BTV). EHDV causes disease in cattle and wild cervids, and has been associated with BTV-like disease in cattle. The virus has important implication for the international livestock trade, and has been isolated from cattle throughout the world (North America, Morocco, Japan, Australia, Israel, Turkey). Differentiation of BTV and EHDV is necessary because detection of infection caused by these viruses is often confused. We have developed two complementary diagnostic tests for EHDV detection: a real-time (rt)RT-PCR and an ELISA. This paper reports (i) the validation of the PCR kit with different EHDV strains, and (ii) the development of a blocking ELISA for serodiagnosis.

The LSIVetMAX EHDV kit is a duplex rtRT-PCR assay using (i) a set of primers and probe labelled with FAM targeting a sequence of the VP6 gene for EHDV detection, and (ii) a set of primers and probe labelled with VIC targeting the beta-actin gene (internal positive control). As an external positive control (EPC), a plasmid obtained by cloning was used. RNAs isolated from 9 different EHDV genotypes and different BTV strains (from different locations world-wide) were used to evaluate specificity and sensitivity. The limit of detection of the EHDV rtRT-PCR was determined by testing a quantified plasmid. All the EHDV strains were detected by the EHDV rtRT-PCR assay. No cross-reaction was identified between the EHDV assay and different specific genotypes of BTV. The detection limit of each PCR is 3 copies of plasmid per PCR.

The LSIVet™ Ruminant EHDV-Serum ELISA uses the VP7 protein to detect EHDV-specific antibodies in serum samples. Due to the antigenic similarity of BTV and EHDV, the sensitivity and specificity of the EHDV assay was evaluated and compared to BTV samples. The sensitivity of the kit was 100% based on a large panel of EHDV positive sera including the 9 existing serotypes. The specificity was >99%. No cross-reactivity with BTV samples was observed. The design of the kit was optimized to match with the most stringent regulations, including a certified serum in the positive control and the conjugate diluent. It shows a high robustness and stability and its fast protocol enables to have results in 55 minutes.

In summary, a combination of ELISA and PCR diagnostic test kits for EHDV should allow improved testing scenarios for epidemiologic purposes.
Comparison between a newly developed ELISA-A/G and some serological tests for the detection of IgG antibodies to *Toxoplasma* in multiple animal species

Batol Al-Adhami, Alvin Gajadhar

Toxoplasma gondii is a zoonotic protozoan parasite that can cause significant disease and losses in livestock and wild animals. Effective control strategies require rapid, reliable, and cost-effective detection methods for large scale surveys and diagnostic applications in a broad range of host species. There is a need for a reliable non-host-specific serologic assay for toxoplasmosis. An indirect ELISA (ELISA-A/G) using protein A/G was developed and evaluated on serum and meat juice samples from a variety of experimentally or naturally infected livestock and wild animals, including pigs, sheep, cats, mice, and wide range of wild animals. Samples were also tested by indirect ELISA-IgG, the modified agglutination test (MAT), and Western blot analysis.

Comparative analysis of test results from samples obtained from experimentally infected pigs, cats, mice, and seals showed excellent agreement among the ELISA-A/G, ELISA-IgG, and MAT. Similar correlation was observed when samples from naturally infected host species were tested by these same assays. Furthermore, a consistent band pattern was present on WB when protein A/G conjugate was used on samples from experimentally infected animals.

ELISA-A/G is a convenient and effective method for serological detection of *T. gondii* infection in multiple host species and is capable of testing samples from a broad range of domestic, wild, and aquatic mammals. The use of protein A/G conjugate in a modified WB assay for confirmation of *T. gondii* infection in mammalian hosts was also demonstrated in this study.
Canada has a strong history of animal health surveillance, recognizing positive benefits to animal health, welfare, and trade. Nationally, the Canadian Animal Health Surveillance Network (CAHSN) continues to evolve. In 2014, government and industry committed to a national “network-of-networks” approach, encouraging further development of synergistic networks involving veterinarians from laboratories, private practice, research, and government. Quebec has enjoyed 22 successful years with their provincial network, Reseau d’Alerte et d’Information Zoosanitaire (RAIZO), upon which the Ontario Animal Health Network (OAHN) was modeled. The Canadian Swine Health Intelligence Network (CSHIN) and the Canadian Animal Health Laboratorians Network (CAHLN) provide examples and a framework for national, collaborative networks.

Within OAHN, an “Expert Network” is being developed for each animal species, comprising specialists from laboratory, academic, and provincial OMAFRA resources, together with 3-4 private practitioners. Expert Networks employ on-line clinical impression surveys of veterinarians working on the species, combined with data from the Animal Health Laboratory (University of Guelph). The networks are tailored to the group, and use existing communication channels (e.g., listserves, VIN, producer newsletters.) In some networks, condemnation data or private laboratory data are also used. Expert Networks are nimble, and the groups can be called together quickly in the event of emergency, or if a risk assessment/expert opinion is required. The group’s quarterly meetings involve translation of data into useful, timely information for the field veterinarian, and determining issues of concern for the industry. Meetings often produce educational opportunities for all parties, and a wide variety of knowledge transfer takes place. The network is a unique opportunity for laboratory, government, and academic veterinarians to improve lines of communication, generate personal contacts, and build trust. The veterinary report includes a summary of data, diagnostic tips, clarification about testing, scientific papers of interest, impending threats, etc. The intent of the report is to share surveillance information while improving lab diagnoses of the veterinarian through awareness of current trends and resources. A producer report is also created with the goal of increasing awareness and recognition of conditions and to trigger animal health conversations with their veterinarian.

Technology is employed to convey and collect information. The program uses social media tools such as Twitter and Facebook to provide up-to-date disease and lab testing information for veterinarians. Podcasting has been a successful knowledge transfer tool, using audio interview recordings for veterinarians on the road. Wet labs, lab tour and lunch talks have also been utilized to improve knowledge and relationships with the laboratory.
036 Developing endemic animal disease surveillance capacity using provincial diagnostic animal health laboratory data

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In Canada, the provincial diagnostic animal health laboratories play an important role in the diagnoses of animal diseases. Historically, apart from certain reportable and zoonotic disease, data generated from most provincial animal health laboratory submissions has not been analyzed and systematically reported. In 2012, the Canadian Animal Health Surveillance Network (CAHSN) Endemic Disease Surveillance Project was initiated in British Columbia (BC) as one of several projects intended to improve capacity for animal disease surveillance using the CAHSN database on the Canadian Network for Public Health Intelligence (CNPHI) platform.

BC elected to focus their initial effort on endemic diseases of poultry and after consultation with poultry veterinarians and pathologists, 3 endemic poultry diseases, reovirus (Reo), Mycoplasma gallisepticum (MG), and infectious laryngotracheitis (ILT) were selected for inclusion. The goal was to use these diseases as ‘test cases’ to initiate the process of data sharing, perform data analysis, generate reports containing synthesized information, and document barriers to performing these activities. Data analysis and synthesis began in January, 2014. At that time, data from two provinces, BC and Manitoba (MB) was automatically uploaded daily from provincial laboratory information management systems (LIMS) to the CAHSN database. There were slight differences between the 2 datasets based on differences in the provincial LIMS; these were documented and data-cleaning routines and queries were built to allow repeatable data analysis. Requests were made to other provinces either to initiate automated data upload of the minimum dataset from provincial LIMS to CAHSN or to provide monthly poultry submission numbers, and total and positive test counts for ILT, MG and Reo to the project team on a quarterly basis. In the first year, we have received data for 1 or more quarters from 6 provinces. For BC and MB, we have corrected minor deficiencies in the fields automatically uploaded to CAHSN to improve data quality and increase the efficiency of retrieving data from CAHSN.

We have created a report template and used this to generate quarterly and annual reports for ILT, MG, and Reo. We have begun to gain an understanding of how passively collected provincial diagnostic laboratory data represents provincial and national poultry flocks and where surveillance gaps exist. We have also documented a number of challenges, including: provincial LIMS that cannot currently support automated data upload to CAHSN; provincial LIMS that are challenging to query for the necessary data for within-province analysis; and lack of resources in some provinces that created a paradox in that staff did not have resources to perform quarterly data queries within province but also did not have resources to initiate an automated data upload so that the project team could perform data analysis.
Established in 2010, the Food and Drug Administration’s Veterinary Laboratory Investigation and Response Network (Vet-LIRN) United States (FDA) coordinates the resources and expertise of 37 veterinary diagnostic laboratories in the United States and Canada to provide the means for rapid response to reports of animal injury from feed.

Vet-LIRN has also been investigating the illnesses in dogs associated with eating pet jerky treats.

Vet-LIRN has managed the work of 11 laboratories to harmonize a method for detecting *Salmonella* in pet fecal samples to evaluate the consequences of contaminated feed on background infection prevalence and to facilitate case investigations.

Vet-LIRN provides several competitive funding opportunities to its laboratories, including infrastructure funding grants to support case investigations and method testing grants to validate network-wide testing methods.

Vet-LIRN has conducted multiple network-wide chemical and microbial proficiency tests to demonstrate that the laboratories provide accurate and meaningful testing data to FDA.
Prairie Diagnostic Services Laboratory Information Management System (LIMS) development experience: a case study

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A steady increase of laboratory data volume together with rising client demands and expectations predicated the decision to upgrade the LIMS at Prairie Diagnostic Services in 2010. Analysis of the products offered on the market for veterinary diagnostic LIMS identified 2 major shortcomings: high cost and insufficient flexibility. From experience of similar facilities in North America, it is almost an inevitable requirement to employ an IT specialist in order to have an “off the shelf” system sufficiently customized and maintained. Based on these findings, PDS made a strategic “build-vs-buy” decision to invest in an in-house LIMS development.

Major requirements to a next-generation LIMS included: full web integration, ease of data retrieval, ability to integrate all real-time analytic instruments in the laboratory, and running on a current standard software platform. Microsoft SQL server 2012 was chosen as the platform for the LIMS database. A real-time integration with Microsoft Dynamics Great Plains financial system supports the invoicing function.

In total, it took approximately 5,000 programming hours over 3 years to develop a system ready for deployment. Two parallel testing sessions (week-long each) were implemented in order to identify major deficiencies and prepare PDS personnel for the big change-over. The new PDS LIMS was successfully launched June 2, 2014. At this time, we are able to say that it is a successful product meeting or exceeding all of the original requirements identified as the project goals.

There is on-going development of add-on services to the system. Next stage goals include creation of web-based submission forms with maximized automatic data capture, inclusion of legacy data into the new database, support for additional real-time data acquisition devices, and automated notifiable disease event reporting.
Where to draw the line - determining cut-off thresholds for Luminex data using mixture-models

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Previously considered an obscure and futuristic technology, bead-based multiplex assays (BBMAs) are becoming accepted as “must have” for veterinary diagnostic laboratories. This is principally on account of their capability to simultaneously detect multiple “analytes” in solution, be they pathogens, antigens, or antibodies (1). This is particularly relevant to the diagnostic situation of a suspect exotic disease, where a number of potential pathogens must be excluded (2). Another diagnostic scenario where multiplex assays have a particular relevance is the detection of potential pathogens in wildlife reservoirs. Bat species have been identified as an important reservoir, and this is the particular focus of our development of a specific multiplex assay platform (“Luminex X-Tag”) to detect novel paramyxoviruses and filoviruses in bat urine collected from Australia and Bangladesh.

In the development and validation of BBMAs, one essential consideration is the cut-off threshold to define diagnostic “positives” and “negatives”. For well-characterized agents, the “gold-standard” is usually considered to be validated quantitative real-time PCR (qRT-PCR) assays. However, in dealing with emerging pathogens, consensus thresholds for defined targets rarely exist.

To develop a threshold in the absence of gold-standard data, we used a data-mining approach based on “finite mixture modelling”. This is a generic framework that identifies different populations by their differing probability distributions. In this case, our 2 populations were animals with samples positive and negative for the individual targets. To fit the 2 mixture distributions, we adapted an approach previously described for biomarkers (3) whereby 2 Gaussian distributions were aligned to the histogram of the natural log of the each of the 2 mean fluorescence intensity (MFI) datasets, viz. for the samples from Australia and Bangladesh. The optimum cut-off was determined as the value where the probability density functions of the 2 distributions coincide. This cut-off needs to be considered presumptive, but provides a focus for follow-on validation work to a restricted sub-sample, thus enabling the determination of a robust threshold in a cost-effective manner.

References
Declining honey bee health levels have led to worldwide losses, and continued losses may jeopardize pollination-dependent food production. Researchers have been unable to identify a single etiological basis for these losses; it may well be the result of the complex interplay of multiple factors. In response, laboratories are beginning to offer services to evaluate those viruses, bacteria, and parasites that may have a negative impact on bee health. But as a nascent field, bee testing has very few guidelines or standards, affecting the quality, applicability, and comparability of results. As part of our testing for 2014, the Animal Health Laboratory determined test sensitivity and assessed testing paradigms including the feasibility of pooling samples from multiple bee colonies and yards to balance sensitivity, meaningful data, and cost considerations.

Sampling bees from a colony is challenging due to the high variance in viral loads, requiring many bees to attain colony representation. Similar results can be achieved by pooling multiple bees in a sample. For example, acute bee paralysis virus (ABPV) levels from 52 individuals from a colony were similar to the mean of 4 groups of 5 bees (3.25E6 ± 5.53E6 (SD) and 1.42E6±7.16E5, respectively, p=0.5). However, pooling large numbers of bees can reduce the sample detection limit (e.g., ABPV: 1.15E4 vs 2.3E5 viral copies for 5 and 20 bees, respectively). Bootstrap analysis shows that sampling multiple small groups of bees can provide results similar to larger groups while maintaining test sensitivity (ABPV mean 2.99E6 ± 1.08E6 for 4 groups of 5 bees, 3.18E6 ± 1.2E6 for groups of 20 bees, p=0.88; detection limits as above). Pooling samples is dependent on the virus in question. Data show that for viruses ubiquitously expressed throughout a yard, pools from multiple colonies are representative of those colonies (e.g. black queen cell virus, 4 individual yards: 1.53E9, 1.21E9, 3.52E8, 2.4E6; Pool: 9.53E8). For some viruses, pooling colonies within a yard prevented the identification of negative colonies when pooled samples were positive (e.g. deformed wing virus: 3/6 colonies). Pooling of colonies from multiple yards also resulted in false-negative colonies (e.g. Kashmir bee virus: 2/12 colonies).

These data show that until meaningful thresholds are established, laboratories should balance test sensitivity, colony representation, and cost considerations. Pooling of colonies should be used only when viruses are ubiquitous, and pooling colonies from multiple yards should be avoided in most instances. Care must be taken to ensure that data are meaningful and representative of colonies and to facilitate the determination of viral thresholds in bee health.

Acknowledgment: This work was funded by the Ontario Ministry of Agriculture, Food and Rural Affairs, Animal Health and Welfare Branch, through the Disease Surveillance Program contract with the University of Guelph.
Rapid and accurate identification of *Streptococcus equi* subspecies by MALDI-TOF MS

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The genus *Streptococcus* includes several very important animal and human pathogens. Among these, *Streptococcus equi* subsp. *equi* is a highly contagious organism, which causes the feared respiratory tract infection “strangles” in horses worldwide. Similarly, *Streptococcus equi* subsp. *zooepidemicus* may cause severe respiratory tract infection in horses and dogs, and is the main cause of uterine infections in mares. Due to great phenotypic and sequence similarity between the 2 subspecies, their discrimination remains difficult.

In this study we aimed to design and validate a novel, Superspectra-based, MALDI-TOF MS (SARAMIS; bioMerieux) approach for reliable, rapid, and cost-effective subspecies-specific identification of *Streptococcus equi* subsp. *equi* and *Streptococcus equi* subsp. *zooepidemicus*, respectively. Superspectra created in this study enabled correct identification of 118 strains of *Streptococcus equi* subsp. *equi* (SEE), *Streptococcus equi* subsp. *zooepidemicus* (SEZ), *Streptococcus equi* subsp. *ruminatorum* (SER), and strains of *Streptococcus dysgalactiae* subsp. *equisimilis*, isolated from various hosts, infection sites, and locations between 1979 and 2014. In general, higher average confidence levels for identification were achieved for SEE (99.2%) compared to SEZ (93.8%). This result may be attributed to the highly clonal population structure and thus a high phenotypic homogeneity in SEE, as opposed to the broad diversity observed in SEZ associated with horses. Importantly, 2 atypical colony variants of SEE and 4 of SEZ encountered among the examined strains, were correctly identified by MALDI-TOF MS. Atypical colony variants are often associated with a higher persistence or virulence of *S. equi*, thus their accurate identification is of high importance.

In conclusion, reliable identification of closely related subspecies of *S. equi* was achieved using an amended SARAMIS database. Additionally, first results on subtyping of SEZ showed that a more refined discrimination, for example for epidemiologic surveys, may be possible.
Rapid and sensitive insulated isothermal polymerase chain reaction on field-deployable POCKIT system enables on-site detection of Equid herpesvirus 1

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Equid herpesvirus 1 (EHV-1) is one of the most important equine viral pathogens that occurs around the world. Clinical outcome of EHV-1 infection in horses varies from acute upper respiratory tract disease, abortion, neonatal death, and neurological disease that may lead to paralysis and a fatal neurological syndrome, equine herpesviral myeloencephalopathy (EHM). Several traditional serologic and virus isolation assays have been described, however, these assays have inherent limitations that prevent rapid and accurate detection of EHV-1. The development of polymerase chain reaction (PCR) based assays has allowed us to detect viral nucleic acid present in clinical specimens precisely and rapidly as compared to the traditional methods that detect the viral agent/antigen or anti-viral antibodies in serum.

Here we describe development and evaluation of a novel, inexpensive, user-friendly assay based on insulated isothermal PCR (iiPCR) method on the POCKIT™, a field-deployable device, for point-of-need detection of EHV-1 in clinical samples. The primers and probes were designed to a highly conserved region of the open reading frame 30 (encodes for the viral DNA polymerase enzyme) to detect both neuropathogenic and non-neuropathogenic strains of EHV-1. Performance of the new EHV-1 iiPCR assay was compared to 2 previously described real-time PCR (rPCR) assays in 2 diagnostic laboratories. Testing serial dilutions of plasmid DNA containing ORF30 sequence revealed the analytical sensitivity of the EHV-1 iiPCR assay to be 16 copies per reaction. Analytical sensitivity of this new assay in detecting viral DNA from both neuropathogenic and non-neuropathogenic strains was shown to be comparable to the reference rPCR assays. The EHV-1 iiPCR assay was highly specific and did not cross react with other equine viral pathogens. Performance of the newly established iiPCR was compared with the rPCR assay in parallel, using DNA isolated from 104 archived clinical samples. All 53 rPCR-positive samples tested positive by iiPCR. Only 46 of the 51 samples negative by rPCR were negative by iiPCR. Overall, compared to the rPCR method, iiPCR had sensitivity of 100% (confidence index 95%: 95.14-100%) and specificity of 90.20% (confidence index 95%: 81.08-99.31%). The agreement was 95.19% (confidence index 90.48-99.90%) with a kappa value of 0.90.

In conclusion, the EHV-1 iiPCR method, working with the field-deployable POCKIT™ device, is a robust tool to provide specificity and sensitivity comparable to rPCR for EHV-1 detection at points of need.
Foot and mouth disease (FMD) is a highly contagious viral disease of cloven-hoofed animals. The differentiation of herds that have been infected from those that have been vaccinated is a critically important follow-up activity to protective emergency vaccination. Both infection and vaccination elicit antibodies against structural antigens, but only assays that measure levels of antibodies against non-structural protein (NSP) can differentiate infected from vaccinated animals (DIVA).

The ID Screen® FMD NSP Competitive ELISA is designed to detect 3ABC non-structural protein (NSP) antibodies in bovine, ovine, caprine, and porcine serum or plasma samples. This study outlines validation data obtained for this ELISA.

Specificity was evaluated on 364 bovine sera, 296 swine sera, 141 sheep sera, and 159 goat sera from non-endemic and non-vaccinated areas (France). Specificity was found to be superior to 99.4%, regardless of the species tested. Agreement between the short and overnight protocols was high (99.69%).

To evaluate sensitivity, the international reference panel of NSP sera used for kit evaluation, comprised of 36 sera derived from vaccinated or unvaccinated and experimentally infected cattle at IAH, Pirbright (1), was tested. Results were kindly provided by ANSES, Maisons-Alfort, France and the Pirbright Institute, UK. All 13 strains present in this panel were correctly detected by the ID Screen® ELISA.

The IAEA serum panel from infected cattle, including 6 FMDV serotypes (A, O, Asia 1, SAT1, SAT2, SAT3) was also tested. The ID Screen® test efficiently detected experimentally infected animals, including carrier animals. All 6 serotypes in the IAEA panel were correctly identified as positive.

In conclusion, the ID Screen® FMD NSP Competition ELISA demonstrates high specificity and excellent performance on reference panels. The ELISA correctly identified all strains tested and efficiently detected carrier animals. The kit offers both short and overnight protocols. These protocols give similar results, meaning that laboratories have the possibility of offering same-day results to their customers if the short protocol is used. The test is applicable to multiple species, including ruminants and swine. Easy-to-use, the kit includes colored and ready-to-use reagents.

Reference
Infectious bovine rhinotracheitis (IBR) is a disease caused by *Bovine herpesvirus 1* (BoHV-1, infectious bovine rhinotracheitis virus, IBRV) which affects domestic and wild cattle across the globe. The infection has been eliminated in several countries in Europe and BoHV-1 eradication and control programs are underway in many other countries worldwide.

Compared to other IBR ELISAs, the lower sensitivity of IBRV-gE ELISAs is a major disadvantage of IBRV control programs based on IBRV marker vaccination. Therefore, IBRV-gE ELISAs are not usually recommended for testing pooled or bulk milk samples. One aim of this study was to compare the performance of the new cattle-type BoHV-1 gE Ab kit (QIAGEN) to that of the HerdChek IBRgE antibody ELISA from supplier I. Another objective was to evaluate the effectiveness of commercially available cattle-type Milk Prep Kit (QIAGEN) at concentrating and purifying antibodies in milk and hence improving the sensitivity of IBRV-gE ELISAs with pooled and bulk milk samples.

QIAGEN’s cattle-type BoHV-1 gE Ab correctly detected all the serum and milk samples of the German FLI reference panel. Based on 249 serum samples from BoHV-1-positive herds, the assay scored a sensitivity of 93.2%, equaling that of the HerdChek IBRgE. Based on 2,985 samples from IBRV marker-vaccinated but non-infected herds, cattle-type BoHV1 gE Ab scored 100% specificity versus a calculated specificity of 99.2% for HerdChek IBRgE. Due to its high sensitivity and excellent specificity, cattle-type BoHV-1 gE Ab is one of only 2 new IBRV gE ELISA kits to pass the strict registration process for official veterinary use in Germany.

A single IBRV-gE-positive cow is likely to remain undetected in a pool of 49 negative milk samples without antibody concentration and purification. The bulk milk concentration procedure improved sensitivity from 5.4% to 75.7% in a positive herd. Milk samples with a very or moderately positive signal are more likely to be detected after pool milk concentration than weakly positive ones. Whereas a follow-up study involving the monthly testing of bulk milk samples from 3 marker-vaccinated IBRV-gE negative herds over a period of 7 months always yielded negative results, milk from a herd containing < 5% IBRV-gE-positive cows were always positive after concentration.

The cattle-type BoHV-1 gE Ab and cattle-type Milk Prep Kit enable improved detection of BoHV-1 infection in serum, milk, and bulk milk samples.
Comprehensive analysis of bovine abortion combining classical and next-generation sequencing approaches

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Abortion in ruminants has significant economic impact in agriculture requiring prompt diagnosis of possible causes. The etiology of abortion can be very complex and the majority of abortions are not epizootic but sporadic. Yet, few infectious agents are examined in routine abortion diagnosis. In microbiome research, next-generation sequencing (NGS) has become a valuable tool in microbial community studies. To improve our knowledge of the bacterial community present in bovine abortion, we studied 33 placentae and 48 fetal abomasa corresponding to 63 cases by Illumina MiSeq 16S rDNA sequencing technology (Microsynth, Switzerland).

DNA extraction was carried out using the QIAamp Mini Kit (Qiagen GmbH) and amplicon sequencing targeted variable regions V3-V5 of the 16S rDNA. Samples were also analyzed by histopathology, bacteriological and mycological culture, by molecular methods for Chlamydia abortus, Coxiella burnetii and Leptospira spp. and for neosporosis, bovine viral diarrhea and Schmallenberg virus.

NGS data analysis identified an average of 30 representative operational taxonomic units (OTUs) for placenta samples (0.3% Actinobacteria, 5.6% Bacteroidetes, 16.3% Firmicutes, 2.8% Fusobacteria, and 74.6% Proteobacteria) and 26 representative OTUs for abomasum samples (0.0009% Actinobacteria, 2.1% Bacteroidetes, 12.3% Firmicutes, 0.006% Fusobacteria, and 85.6% Proteobacteria). In both organs, Pseudomonaceae was the most abundant family. The results revealed opportunistic pathogens of interest such as Campylobacter fetus subsp. fetus, Trueperella pyogenes, Streptococcus pluranimalium. PCR was negative except from one case of C. burnetii. In 20 cases, we found a virological cause (BVDV n=3, Schmallenberg n=11) or parasitological cause (Neospora caninum n=6). All cases of neosporosis and one case of BVD, with co-infection with S. pluranimalium, showed necrotizing placentitis. Broad spectrum culture revealed a possible bacterial or fungal agent in 23 cases; however, only 9 cases were associated to necrotic placentitis (Aspergillus fumigatus n=1, Candida sp. n =1, C. fetus subsp. fetus n=1, E. coli n =2, S. pluranimalium n =2, S. uberis n=2). Overall, broad-spectrum analysis could attribute a possible cause of abortion in 46% of the cases.

Increased knowledge of the microbiome involved in abortion offers new perspectives in diagnosis, e.g. the implication of Pseudomonaceae, which are known to be possible opportunists in abortion, but have not been studied in detail. The placenta remains an understudied organ and amplicon sequencing data have the potential to shed light on the relation between placental and fetal microbiome. NGS should be extended to other microorganisms such as fungi. Nonetheless, to date classical diagnostic methods are still needed to detect specific abortive agents.
046 Real time PCR with melt curve analysis as a novel screening tool for coccidia of veterinary importance

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Simple, robust, and reliable diagnostic methods are essential for management of coccidiosis in food animals, pets, and wildlife. Our objective was to develop a simple screening tool for multiple species of coccidia in a variety of matrices including feces, tissues, food products, and environmental samples. We designed a real-time quantitative PCR assay with melt curve analysis (qPCR MCA) to sensitively detect, rapidly identify, and reliably differentiate DNA from coccidia species of various host species such as cattle, poultry, swine, canids, and felids. A universal primer cocktail was developed to amplify 18S rDNA from virtually any coccidia species using qPCR with fluorescent dye detection, followed by MCA to identify each species by their unique melting curves and differentiate them based on melting temperature (Tm).

The assay has been validated for the detection of Cyclospora, Toxoplasma, and Cryptosporidium oocysts and is currently being used to test fresh produce in food safety surveys. It has also been successfully utilized in a public health survey to test fecal samples for detection of Cystoisospora, Cryptosporidium, and Cyclospora in humans. Further customization and applications of the qPCR MCA are continually being explored. This assay has the potential to detect previously uncharacterized or unknown species of coccidia. For example, when applied to arctic fox fecal samples collected in Karrack Lake, Nunavut, Canada, the assay detected unique lineages of Sarcocystis (16/95), Eimeria (8/95), Cystoisospora spp. (5/95), and Neospora/Hammondia spp. (1/95). Using gDNA samples from oocysts, the assay can differentiate selected Cryptosporidium and Eimeria isolates to the species level which could have significant benefits for the management of bovine and avian coccidiosis. This screening tool has also been applied successfully for the detection of tissue coccidia Toxoplasma, Hammondia, Neospora, and Sarcocystis.

When used with proper controls and adequate validation for the intended purpose, the qPCR MCA assay can be used to screen DNA samples from feces, fresh produce, meat, or water, and provide evidence of known, unknown, or emerging coccidia species in various sample matrices.
Addition of PCR methods to conventional serology for the routine diagnosis of equine piroplasmosis

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Equine piroplasmosis is a tick-borne protozoal infection generally difficult to diagnose clinically as clinical signs are non-specific. The infection also represents an important constraint to the international movement of equids. Common practice is the sole use of serologic tests for piroplasmosis, and for those available there is limited data on their validation. Reliable laboratory tests or their combination are essential. In a previous comparative study, real-time (RT) PCRs targeting the rRNA 18s gene (1,2), 1 for each parasite, were adopted on the basis of their optimal performance. This study presents and discusses their use in conjunction with the OIE-prescribed serologic tests: ELISA (VMRD, USA) and indirect immunofluorescence (IFAT, Fuller Laboratories, USA) to analyze blood samples from 274 horses. For comparison of the results, the PCR was used as the reference method since sequencing of derived amplicons confirmed these as T. equi and B. caballi. Case definition for an acute form of piroplasmosis was temperature >38°C and at least one of the following signs, jaundice, anemia, and petechial hemorrhages, and a PCR positive (+) result.

The B. caballi RT-PCR detected 14 + samples, none confirmed in ELISA, while the IFAT had a sensitivity (Se) of 50% (7/14) and a specificity (Sp) of 87.7 % (228/260). The T. equi RT-PCR detected 137 + samples with the ELISA showing a Se of 67.2% (92/137) and a Sp of 83.2% (114/137) and the IFAT, a Se of 86.1% (118/137) and a Sp of 81% (111/137). For both parasites, the IFAT, even if still limited, presented an apparently major sensitivity when compared to the ELISA, but liable to cross-reactivity. Results for B. caballi are preliminary, due to the limited number of + samples recruited.

Of the PCR + horses for B. caballi and T. equi, only 28.7% and 19.7% respectively were defined as cases, possibly due to the persistence of parasites beyond the acute form. In particular, for T. equi the serological tests showed a high agreement and a relatively high specificity. In this circumstance, the PCR negative samples could be due to the sterilization of the infected horses, occurring spontaneously or following treatment. With the further introduction of quantitative PCR, treatment efficacy could be monitored especially in view of the side effects these possess. This study demonstrates that the simultaneous use of PCR with serologic tests increases the diagnostic probability to define the sanitary state for equine piroplasmosis for the purposes stated above.

References
Increasing standardization and throughput in real-time PCR using a modular system

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Although real-time PCR has offered significant advantages over end-point PCR, commercial real-time PCR assays still use a set of reagents, or “kit”, designed for testing a precise number of samples for a specific target(s). This approach often requires a separate testing protocol for each target, causing longer time to results and more hands-on time for laboratories.

IDEXX RealPCR™ real-time PCR reagents aim to provide a new level of standardization to PCR diagnostics by using reagents in a modular system. The components are shared over the entire platform, making it possible to run any specific detection mix with a standard master mix and a single positive control. Moreover, the system maintains a single PCR program for all DNA and RNA targets.

The first detection mixes on the RealPCR modular system include primers and probes for bovine viral diarrhea virus (BVDV) and swine enteric coronaviruses. For swine enteric coronaviruses, 4 sets of reagents are available: PEDV (porcine epidemic diarrhea virus), TGEV (transmissible gastroenteritis virus), PDCoV (porcine deltacoronavirus) as well as a multiplex for PEDV and PDCoV.

The RealPCR BVDV reagents detect type I, type II, and type III (Hobi). The test shows an analytical sensitivity of $\leq 15$ copies/reaction for type I, type II, and HoBi. The test displays no cross-reactivity with many common bovine viral pathogens either by in silico analysis or with diagnostic specificity testing.

All PEDV and PDCoV reagents have been tested with oral fluid and fecal swab samples and each demonstrates $>97.7$ % sensitivity and specificity for the samples tested to date. The swine enteric coronavirus assays maintain analytical sensitivities of $\leq 10$ copies per reaction even in the presence of an artificially high concentration of the internal control. Compatibility with the IDEXX RealPCR modular system allows these assays to be “mixed and matched” according to specific testing needs and run side-by-side with other RealPCR reagents maintaining fast run protocols and increasing laboratory workflow efficiency.

To ensure reliable results, the reagents employ a multiplexed internal sample control (ISC) that eliminates the need of an internal positive control (IPC) spike and provides a control for all steps in the PCR workflow. The RealPCR Quality Control reagents provide additional tools for environmental contamination monitoring programs. The RealPCR QC Signature Detection Mix specifically identifies positive control contamination in laboratories, while the RealPCR QC Pan Bacterial Detection Mix is useful as a positive control when running environmental control samples.

The use of shared reagents and standardized testing over the modular system greatly increases efficiency, decreasing real-time PCR turnaround times for laboratories. Combining different assays in a single PCR run overcomes the issue of not having enough samples to justify a PCR run.
049 Development of an alternative artificial digestion method for detecting *Trichinella* larvae in meat of domestic swine

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According to Regulation (EC) No. 2075/2005 (European Community, 2005), carcasses of domestic pigs, horses, wild boar, and other farmed and wild animal species susceptible to *Trichinella* infestation should be systematically sampled in slaughterhouses or game-handling establishments as part of the postmortem examination. The magnetic stirrer method for pooled sample digestion is considered as the reference method of detection of *Trichinella* larvae for routine use. This reference method is based on pepsin, as digestive protease to release the *Trichinella* larvae from the muscle meat, which are then identified under a microscope. In recent years, compliance with the EU regulation has been hampered by frequent supply shortages of pepsin and variations in quality. Moreover, the use of pepsin powder and hydrochloride acid is a potential work safety issue. Therefore, an alternative artificial digestion method, the PrioCHECK® Trichinella AAD, has been developed and is now officially recognized as an equivalent method for artificial digestion by the Commission of the European Communities. With a standardized production of a recombinant proteinase, liquid components, and a digestion performed at a slightly basic pH, the PrioCHECK® Trichinella AAD has clear advantages over the pepsin-based digestion method.

The PrioCHECK® Trichinella AAD is comprised of 3 components, (1) digestion buffer, (2) digestion buffer additive, which provides an optimal digestion environment for the enzyme, and the (3) enzyme solution. The digestion procedure is comparable to the reference method (magnetic stirrer method) and allows pooling of individual meat samples up to 100 g followed by digestion at 60°C for 20 min under constant stirring.

The performance of the PrioCHECK® Trichinella AAD was evaluated in >70 digestion runs, including diaphragm samples from slaughterhouses, experimentally infected animals, and samples spiked with *Trichinella* larvae obtained from the European Reference Laboratory for Parasites (EURLP). The digestion process was demonstrated to be fully compliant with the guidelines requiring the digestion residue that remains on the sieve to be below 5% of the starting meat tissue. All spiked samples were correctly classified as positive, and the method was found to consistently identify 1-3 larvae in samples spiked with 3 *Trichinella* larvae. During the digestion process, the *Trichinella* larvae are inactivated but they are morphologically intact and can be used for further strain typing by PCR.

The PrioCHECK® Trichinella AAD was subjected to the validation process of the EURLP and recommended as equivalent method for detection of *Trichinella* in meat of domestic swine.
Molecular detection and genetic diversity of *Anaplasma marginale* and *A. marginale* subsp. *centrale* in cattle in South Africa

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Bovine anaplasmosis, caused by *Anaplasma marginale*, is a globally prevalent virulent hemoparasite of ruminants. In South Africa, it is endemic in most of the cattle-farming areas. *A. marginale* subsp. *centrale* causes a milder form of anaplasmosis in cattle and has been used as a vaccine in many parts of the world. However, this vaccine has major drawbacks and therefore a safer and more effective vaccine is required. In order to examine *A. marginale* candidate genes for the development of an efficient recombinant vaccine, a bank of *A. marginale*-positive field samples must first be established. It is known that many strains of *A. marginale* are present in South African cattle; it is therefore essential to make use of an assay that is able to detect all strains. The aim of this study was therefore to evaluate 3 molecular assays in detecting *A. marginale* and *A. marginale* subsp. *centrale* in cattle in South Africa.

A total of 528 cattle samples originating from 9 provinces in South Africa were analyzed for the presence of *A. marginale* and *A. marginale* subsp. *centrale* using a duplex quantitative real-time PCR (qPCR) assay. The assay detects the msp1β gene of *A. marginale* and groEL gene of *A. marginale* subsp. *centrale*. The results of the qPCR assay were compared to those of the reverse line blot (RLB) hybridization assay, and a nested-PCR assay. The qPCR assay detected *A. marginale* and *A. marginale* subsp. *centrale* in 50% and 17.2% of the samples, respectively. A total of 15.3% of samples had mixed infections. The qPCR assay detected *A. marginale* and *A. marginale* subsp. *centrale* in more samples than the RLB assay, suggesting that the qPCR assay is more sensitive. The level of agreement between the qPCR and nested-PCR assays for detection of *A. marginale* and *A. marginale* subsp. *centrale* was ‘moderate’ (kappa score: 0.597) and ‘good’ (kappa score: 0.757), respectively. Thirty-five samples that tested positive using the *A. marginale* qPCR tested negative using nested-PCR. To investigate these discrepancies, and to determine if the qPCR primer and probe target regions are conserved, *msp1β* and *groEL* genes from 7 samples were amplified, cloned, and sequenced. The extent of the *msp1β* and *groEL* gene sequence variation in field samples was determined using next-generation amplicon sequencing (NGAS). Sequencing results showed variation in the region where the nested PCR internal forward primer would anneal in South African *msp1β* sequences, resulting in many false negatives. NGAS revealed that the *groEL* qPCR primer and probe target sequences were well conserved in field samples containing *A. marginale* subsp. *centrale*. However, the *msp1β* qPCR primer and probe areas were shown to contain SNPs.

It is recommended that a new assay based on a more conserved gene, or a conserved region of the *msp1β* gene should be developed since SNPs may affect the specificity of the current assay in the detection of different *A. marginale* strains in South Africa.
Detection of bovine *Trichomonas foetus* in the USA; PCR results of an 18 lab quality control (QC) panel mimicking field samples with InPouch™ TF and TF-Transit Tubes

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Known colloquially in the USA as ‘Trich’, bovine trichomoniasis is a sexually transmitted disease caused by the flagellated protozoan *Trichomonas foetus*. Endemic to the States west of the Mississippi River, the disease is also prevalent in many other parts of the world and correlates with open-range breeding. Control and management of the disease requires proficient laboratory detection of *T. foetus*, the optimization of which can be complicated by various sampling methods, transportation temperatures, transport media, and methods of DNA extraction with regards to PCR Performance.

Based on the recommendation of the USAHA Trich subcommittee, 18 labs from 16 States came together to determine the level of consistency in PCR detection of bovine *T. foetus* through the creation of a quality control (QC) panel. In order to mimic field samples, each panel consisted of either 20 InPouch™ TF tests or TF-Transit Tubes spiked with 500 µL of *T. foetus*-negative bull smegma. Each panel of 20 samples also contained 10 *T. foetus*-positive samples; duplicates of 5 starting cell concentrations —1,120/224/112/56/11—quantified on a hemocytometer. The samples were organized and prepared by Biomed Diagnostics, and the combined results were coded so that the reported performance of each individual lab remained anonymous. A total of 14 InPouch™ TF and 14 TF-Transit Tube panels were prepared and sent overnight to the 18 participating laboratories for PCR assay.

Of the 11 labs that received pouches, 6 labs identified all positive pouches as PCR positive. Of the 11 labs that received tubes, 5 labs identified all positive tubes as PCR positive. Results were used to have an open discussion amongst the participating labs focused on variations in PCR methodology and general ways to improve the laboratory detection of *T. foetus*. 
052  Serology as a new tool in the control of worm infections in pigs

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Ascaris suum is currently the only parasitic worm that is still highly prevalent in intensive pig production systems. However, due to the subclinical nature of the disease, infection often remains undiagnosed. This creates a lack of information regarding the worm-status of a farm, which ultimately makes it difficult to evaluate the applied deworming programs. Recently, a serodiagnostic test was developed and evaluated that can be used to measure parasitic exposure of fattening pigs more accurately. The test is based on the recognition of a hemoglobin protein (AsHb) produced by the parasite leading to a systemic IgG antibody response in exposed animals. In experimentally infected pigs, the levels of antibodies determined by ELISA have been shown to have a diagnostic sensitivity and specificity of almost 100%. Further evaluation of the test under field conditions have demonstrated a superior sensitivity for the detection of A. suum infections in comparison to fecal egg counts and liver white spots.

A recent seroprevalence study performed with this test on 471 farms across several European countries confirmed the high prevalence of A. suum in fatteners, with more than 50% of the farms analyzed testing positive. Finally, to assess the association between Ascaris and farm productivity, ELISA results of 20 fattening farms were compared with slaughterhouse data (such as percentage of affected livers, pleuritis, and lung lesions) and farm performance parameters (such as feed conversion efficiency, days to market, daily weight gain, carcass quality, and mortality). The results demonstrate a strong correlation between serology and production parameters, such as days to market and average daily growth, further indicating that A. suum infections could have a significant impact on farm economical parameters.

The outcome of these studies show that the Ascaris ELISA is an easy-to-use and sensitive tool that can be used to both assess exposure of fattening pigs to A. suum, estimate the economic losses due to this parasite, and monitor the efficacy of anthelmintic treatment programs. Additional studies are being performed in collaboration with Boehringer Ingelheim Svanova to further optimize this assay for commercial use.
053 An MFIA for the detection of antibodies to PRRSV, SIV, and PCV2

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Porcine respiratory disease complex (PRDC) is a significant economic problem for the swine industry. This syndrome is caused by the interaction of multiple noninfectious and infectious factors, including PRRSV, SIV, and PCV-2. Measurement of antibodies to these viruses is routinely performed for monitoring herd status or optimizing vaccination protocols.

We have developed a 4-plex multiplexed fluorometric immunoassay (MFIA) to detect and differentiate antibodies to PRRSV type 1 and 2, SIV type A, and PCV-2 antibodies.

**Antigens:** Recombinant nucleoproteins (NP) from PRRSV type 1 and 2, avian influenza virus (AIV), and PCV-2 were expressed in *E. coli* (PRRSV, PCV-2) or baculovirus (SIV).

**Ag coupling:** PRRSV type 1 and 2, AIV, and PCV-2 antigens were coupled to 4 different sets of magnetic beads (Magplex®, Luminex) using a proprietary method.

**1-plex MFIA:** all incubations were done at room temperature in the dark on a rotating shaker. Serum samples were diluted and incubated with an equal volume of PRRSV type 1 and 2, AIV, and PCV2 antigen-coated bead suspension. After thorough serial washing, diluted biotinylated goat anti-swine IgG was added to each well and the plates were incubated. The plates were further washed prior to adding streptavidin–horseradish peroxidase (SA-PE) to each well and incubated. After final washing, the beads were resuspended and the plates were read.

**4-plex MFIA:** once the 4 1-plex assays worked properly, the 4 bead suspensions were mixed and used in a 4-plex assay.

**PRRSV, Influenza A, and PCV-2 ELISA:** samples were also tested using the PRRS X3 Ab, the Influenza A Ab (IDEXX, USA), and the Ingezym Circo IgG (Ingenasa, Spain) ELISA kits.

**Test evaluation:** the MFIA were evaluated for sensitivity and specificity using serum samples of known status.

- **PRRSV type 1:** the sensitivity of both the MFIA type 1 and the ELISA was 100%.
- **PRRSV type 2:** the sensitivity of the MFIA type 2 and the ELISA was 95.00% and 96.7% respectively.
- **PRRSV types 1 and 2:** the specificity of the MFIA and the ELISA was 98.89% and 100% respectively.
- **SIV:** the relative sensitivity and specificity of the MFIA vs. the ELISA were 93.13% and 97.44% respectively.
- **PCV2:** the relative sensitivity and specificity of the MFIA vs. the ELISA were 75.53% and 79.50% (agreement: 77.36%)

This preliminary version of a 4-plex MFIA for PRRSV type 1 and 2, SIV and PCV2 has demonstrated promising performance. The agreement with the ELISAs was excellent for PRRSV and SIV but poor for PCV-2. This lack of correlation has been observed previously with other PCV-2 assays. Once optimized, the 4-plex MFIA will be a useful tool for diagnosing and controlling the infections caused by these agents.
An MFIA for the detection of antibodies to multiple APP serotypes

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*Actinobacillus pleuropneumoniae* (APP) remains an important swine respiratory pathogen. Fifteen APP serotypes corresponding to 9 serogroups have been identified so far (APP1-9-11, APP2, APP3-6-8-15, APP4-7, APP5, APP10, APP12). The surveillance of swine herds for APP relies mostly on the detection of serotype/serogroup-specific antibodies in serum samples. Various serologic assays have been developed for that purpose. The most sensitive and specific are indirect ELISA using highly purified long-chain lipopolysaccharides (LC-LPS) as antigen.

We have developed a 7-plex multiplexed fluorometric immunoassay (MFIA) using LC-LPS as antigen to detect and differentiate antibodies to APP1-9-11, APP2, APP3-6-8-15, APP4-7, APP5, APP10, and APP12 in a single assay.

**Antigen coupling:** highly purified LC-LPS APP antigens were coupled to 7 different sets of magnetic beads (Magplex®, Luminex) using a proprietary method.

**1-plex MFIA:** all incubations were done at room temperature in the dark on a rotating shaker (600 rpm). Serum samples were diluted and incubated with an equal volume of the different coated-bead suspension. After thorough serial washing, diluted biotinylated goat anti-swine IgG was added to each well and the plates were incubated. The plates were further washed prior to adding streptavidin-horseradish peroxidase (SA-PE) to each well and incubated. After final washing, the beads were resuspended and the plates were read.

**7-plex MFIA:** once the various 1-plex assays worked properly, the 7 bead suspensions were mixed and used in a 7-plex assay.

**APP ELISA:** samples were also tested using the various APP serotype/serogroup-specific LC-LPS ELISA kits (Swinecheck APP®, Biovet).

**Test evaluation:** the various MFIA were evaluated for sensitivity and specificity using serum samples of known APP status. The relative sensitivity and specificity of the 7-plex APP MFIA were 95 to 100% of those of the corresponding serotype/serogroup specific LC-LPS ELISA.

This preliminary version of a 7-plex MFIA for APP1-9-11, APP2, APP3-6-8-15, APP4-7, APP5, APP10, and APP12 has demonstrated promising performance. Once optimized, the assay will be a useful tool for diagnosing and controlling the infections caused by these agents.
Fast and reliable diagnostics in veterinary microbiology plays an important role in the battle against animal infectious diseases. It is thus of great clinical interest to define an approach that allows rapid and efficient identification of pathogens in order to implement appropriate measures for the benefit of animal and public health.

Even though tremendous progress has been made in the field of molecular biology in the past decade, conventional PCR is still considered a basic and reliable tool in molecular diagnostics. This holds true also for several tests performed in the Laboratory for Molecular Bacteriology of the Veterinary Faculty Ljubljana. To improve the quality of the analysis, traceability, and reduce time, the QIAxcel Advanced system was introduced for separation and analysis of single or multiplex PCR products.

Since 2010, we have successfully identified a number of pathogens using the QIAxcel system (e.g., STEC, Campylobacter sp., Clostridium perfringens, Clostridium difficile, MRSA, Mycobacterium avium, Brucella sp., ESBL-producing bacteria, Listeria monocytogenes, and Taylorella equigenitalis). All the analyses were performed with QX DNA Screening Kit and for the most optimal results AM420 method was selected, with the following electrophoresis parameters: alignment marker injection at 4kV for 20 s, sample injection at 5 kV for 10 s and separation at 5 kV for 420 seconds. QX alignment markers 15bp–1kb or 15bp–3 kb and DNA size markers 50 bp–800 bp and 50 bp–1.5 kb were run simultaneously. During implementation of the QIAxcel system and for each new PCR assay, QIAxcel was always run simultaneously with the classical agarose gel electrophoresis.

After careful optimization of parameters, the QIAxcel system, supported by the QIAxcel ScreenGel software, yielded accurate and reproducible results. Hands-on time was reduced to a minimum: from about 2 hours to 20 minutes for 12 samples. In addition, the analysis of results was less subjective; a good example is the molecular serotyping of L. monocytogenes, based upon 6-plex PCR, where the electropherogram superposition offered by the software enables reliable comparison with positive controls, especially for the amplicons of low intensity.

Based on the data of about 600 runs during the last 3 years, we can conclude that the QIAxcel system definitely minimizes manual intervention, increases work safety and provides better traceability of the data. As the associated software also reduces turnaround time by automated analysis and identification of pathogens, the QIAxcel system makes an important contribution to improved veterinary diagnostics.
Shiga toxin–producing \textit{E. coli} (STEC) accounts for \( \approx 175,000 \) human illnesses annually in the United States. The major STEC serogroups that cause human infections are O157, O26, O103, O111, O121, O45, and O145, also known as “top-7” STECs. Four major virulence factors, Shiga toxin 1 and 2 (stx1, stx2), intimin (eae), and enterohemolysin (ehxA), are commonly associated with severe human infections. STEC cells are harbored and propagated in the gastrointestinal tract in cattle and are shed in feces, serving as a major source of food and water contamination. An efficient and reliable screening tool is important to reduce STEC food contamination.

We have developed an 11-gene multiplex PCR assay using modified QIAGEN Multiplex PCR Plus Kit (cat. #: 206152) and the QIAxcel system that detects the 4 major virulence factors, and detects and differentiates the “top-7” STECs in high-throughput settings. The assay was designed in such a way that the amplicons of the 11 genes are well separated and easily distinguishable on both agarose gel and the QIAxcel electrophoresis system. A total of 185 enriched cattle fecal swab pools (5 swabs per pool) were amplified for 11-genes described above. PCR products were directly run on QIAxcel without further manipulation. Positive amplifications of each of the 11 genes in every sample were manually interpreted and compared with the data generated automatically by the Peak-Calling function in the QIAxcel software. Bands that were manually identified were 98.9\% the same as those identified by Peak-Calling interpretations. Most samples (86.5\%) were positive for at least 1 of the 7 O-types, and 2 virulence genes. The most prevalent \textit{E. coli} serogroup was O157 (61.6\%), followed by O26 (28.1\%), O103 (19.5\%), O121 (18.9\%), O45 (17.3\%), O111 (8.1\%), and O145 (2.7\%). There were 25 samples (13.5\%) that were negative for the top-7 serogroups. \textbf{The overall prevalence of the 4 virulence genes is very high}, with 99.5\% positive for ehxA, 90.8\% for stx2, 89.2\% for eae, and 67.6\% for stx1. The high virulence gene prevalence may partially be contributed to by pooling of the samples.

\textbf{Applying the Peak-Calling method post 11-gene multiplex PCR will significantly save personnel time and will reduce potential interpretation errors generated by manual interpretations.}
On-site (point-of-need, PON) testing has been established for several decades. Due to their high ease-of-use, qualitative lateral flow tests have been the predominant format for infectious disease testing in resource-limited or non-laboratory settings, such as farms, vans, or jeeps. However, depending on the pathogen and the specific need (e.g., pathogen identification and subtype differentiation), rapid molecular testing with a low limit of detection and high multiplexing capacity is more appropriate. The conventional complex molecular workflow comprising sample preparation, nucleic acid amplification (NAA), and detection cannot be transferred to remote settings – its simplification for non-professional users is essential.

In recent years, several isothermal NAA technologies have been developed that have demonstrated relatively high robustness against inhibitory substances. These technologies enable battery powered yet rapid amplification of specific DNA without the need for sophisticated sample purification technologies. However, RNA-based molecular analysis is still hampered by rapid degradation of the analytes by RNases inherent in the sample.

Here we describe the development of a simple modular molecular PON system based on the following workflow: 1) manual centrifugation-free nucleic acid extraction using magnetic particle technology and a few simple steps, 2) reverse transcriptase thermophilic helicase dependent amplification (RT-tHDA), and 3) real-time fluorescence detection using the ESEQuant TS2 Reader.

This portable system has demonstrated robust and efficient viral RNA detection in less than 40 minutes. Future work will focus on full process automation to allow truly simple molecular testing.
RAPID-bTB: Rapid and specific detection of *Mycobacterium bovis* in veterinary diagnostic specimens by a novel lateral flow immunochromatographic assay

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Millions of pounds have been spent on a bovine tuberculosis (bTB) eradication program in the United Kingdom over recent years and yet the prevalence of the disease in the cattle population remains high. In the UK context, the Eurasian badger (*Meles meles*) represents a wildlife reservoir of *Mycobacterium bovis* infection, and transmission of the bacterium between badgers and cattle is likely to be contributing to some bTB herd breakdowns. We have developed an immunochromatographic, lateral flow assay (LFD) that has been shown to be specific for *M. bovis*, and unique in its ability to differentiate between *Mycobacterium tuberculosis* and *M. bovis*. The RAPID-bTB LFD assay is a very simple-to-use test format, provides a result within 15 min, and has a limit of detection for whole *M. bovis* cells of $1.68 \times 10^4$ CFU/mL of MGIT™ culture or badger fecal homogenate.

We present results of 2 trials assessing the potential of the LFD: 1) to directly detect *M. bovis* in badger feces in the field, and 2) to quickly confirm isolation of *M. bovis* in BACTEC™ MGIT™ liquid cultures of cattle lymph nodes in a statutory TB laboratory setting. The novel LFD test (RAPID-bTB), used in combination with immunomagnetic separation (IMS), detected *M. bovis* in 17.7% (78/441) badger feces samples collected from latrines at 110 setts throughout Northern Ireland; compared to 31.7% of same samples testing positive for *M. bovis* by IMS-PCR. As expected, the LFD was less sensitive than PCR, only detecting badgers shedding high numbers of *M. bovis* in their feces, which, it could be argued, are the most important animals in terms of TB control. However, when used to test MGIT™ cultures of cattle lymph nodes as they indicated growth positive on the MGIT 960 system, the novel LFD test was found to have considerable advantage over the currently used methods for confirmation of *M. bovis* in MGIT™ cultures (i.e., Ziehl-Neelsen staining plus spoligotyping) in terms of speed, simplicity and cost. Detection sensitivity and specificity of the novel *M. bovis* LFD compared to the spoligotyping result were calculated to be 1.000 and 0.917, respectively. The latter specificity value is explained by the fact that the LFD test detected an additional 9 *M. bovis*-positive cultures compared to the statutory culture protocol.

The results of these trials clearly demonstrate the potential bTB diagnostic value of the novel RAPID-bTB LFD assay.
Evaluation of an improved commercial *Anaplasma* antibody competitive enzyme-linked immunosorbent assay in cattle in Australia

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A commercially available *Anaplasma* species antibody competitive enzyme-linked immunosorbent assay (cELISA)\(^a\) was recently modified by replacing the recombinant major surface protein 5 (rMSP5)-maltose binding protein (MBP) fusion protein with a rMSP5-glutathione S-transferase (GST) fusion protein, improving diagnostic performance (1). The current study evaluated the rMSP5-GST cELISA for the serologic detection of *Anaplasma marginale* and *Anaplasma centrale* antibodies in cattle in Australia.

The rMSP5-GST cELISA was performed on 275 *Anaplasma*-negative sera with diagnostic specificity of 98.5%, based on the established cut-off of 30% inhibition. The commercially available rMSP5-MBP cELISA, when tested with the same set of sera, had a diagnostic specificity of 71.6%. The *Anaplasma*-negative sera used for evaluation were obtained from cattle sourced from an *A. marginale* non-endemic region, where *Rhipicephalus (Boophilus) microplus*, the only tick vector of *A. marginale* in Australia, is absent. In addition, these cattle had no previous *A. centrale* vaccination history.

Both cELISAs had a diagnostic sensitivity of 100% when tested on 202 experimentally infected *Anaplasma*-positive sera (96 *A. marginale*-positive and 106 *A. centrale*-positive), defined by *Anaplasma* real-time polymerase chain reaction.

This study demonstrates that the rMSP5-GST cELISA has an improved diagnostic specificity and comparable diagnostic sensitivity compared with the commercially available rMSP5-MBP cELISA. The rMSP5-GST cELISA will be a useful serodiagnostic tool for the detection of antibodies to *A. marginale* and *A. centrale* in cattle in Australia in epidemiologic studies and for disease or disease-free certification.

\(^a\) Anaplasma Antibody Test Kit, cELISA; VMRD Inc., Pullman, WA, USA.

Reference
Earlier and easier diagnostic tools for PRRSV herd management: comparison of sampling and prevalence under field conditions

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Real-time RT-PCR (rtRT-PCR) and ELISA tests are often used to assess the PRRSV infection status of pig herds. rtRT-PCR tests on blood/serum and tissue samples are the most used technique to detect PRRSV earlier. Recently, the detection of many swine pathogens in oral fluids has been reported as an alternative technique, being more economical, easier, and less invasive. The main goal of several studies was to validate oral fluid against blood/serum and tissue and also to establish a sampling recommendation for oral fluids under field conditions for earlier detection of PRRSV with an easier sampling method.

Thermo Fisher Scientific requested several laboratories and research institutes throughout the world to evaluate the rtRT-PCR tools on over 800 field samples from different genotypes. A field study in Spain allowed evaluation of the performance of the kit on oral fluid samples. Results per pen were compared to individual results (blood/serum). Based on these results, a biostatistical/epidemiologic study was launched to calculate the probability of genotype 1 and 2 virus detection in a pen using oral fluid samples taking into account the prevalence of PRRSV in serum as an independent variable. In all these studies, sample extraction was carried out with the MagMAX™ Pathogen RNA/DNA Kit (5X) or the MagVet™ Universal Isolation Kit. Purified RNAs were analyzed by rtRT-PCR with the LSI VetMAX™ PRRSV EU/NA kit or VetMAX™ NA and EU PRRSV Reagents.

The LSI VetMAX™ PRRSV EU/NA kit showed a sensitivity of 98.2% on more than 400 positive field samples and a specificity of 100% on more than 400 negative field samples. In the second study, the kit showed an excellent correlation at pen level (oral fluid sample) compared to animal level (blood/serum sample) with a difference of +/-1CT. The PRRSV RNA was identified in early infectious stages compared to antibody detection: from 7 days after infection up to 7 weeks on oral fluids and 8 weeks on blood/serum compared to 28 days after infection with ELISA. The number of oral fluids that need to be sampled on a herd level in order to find at least 1 positive PRRSV oral fluid was for example 3, for a serum prevalence of 50%.

Ready-to-use rtRT-PCR tools allow detection of the virus in the early stage of infection compared to antibody detection. rtRT-PCR results obtained with blood/serum and oral fluids demonstrate excellent correlation. Based on epidemiology and prevalence of the virus in the herd, oral fluid sample are able to provide the same if not better information due to ease of use compared to randomly taken blood samples on the herd level. Oral fluids allow the swine industry to generate a more accurate and reliable monitoring system where early detection can result in faster response time. It is an opportunity to increase the number of pigs tested while decreasing the cost of analysis and to estimate the circulation of pathogens (PRRSV, PCV2 and SIV) in swine populations for effective herd health monitoring.
061 A broadly applicable solution strategy to control PRRSV

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PRRSV outbreaks are feared in the swine industries because of the devastating economic impact. Even though various diagnostic tests for PRRSV including ELISA and PCR systems exist, correct result interpretation in respect to the actual PRRSV status pose a major challenge. Noteworthy are the genetic diversity of PRRSV isolates, the prolonged PRRSV persistence, and the complex immunological behavior of the virus (1). Vaccination against PRRSV has been shown to be an effective tool to reduce clinical disease, however PRRSV infection is not prevented. Vaccine virus may be shed and transmitted to non-vaccinated contact pigs (2), and vaccine virus can persist in boars and be disseminated through semen (3).

The complex nature of the PRRS disease indicates that single diagnostic tests may not be enough to successfully manage PRRSV but rather comprehensive solution strategies are needed for the effective control of the disease.

Here we present a broadly applicable concept for PRRSV disease management using the ELISA PrioCHECK PRRSV VIA. PrioCHECK PRRSV VIA is an indirect ELISA detecting neutralizing antibodies. Combining the results of PrioCHECK PRRSV VIA with herd and site information, RT PCR results as well as PRRSV screening ELISA results can:
- Discriminate type I from type II on herd level
- Detect single or mixed infections of different PRRSV types on herd level
- Assess the chronological order of different infections
- Estimate the time of infection and the virus load based on the serological titre level
- Help to decide over the optimal type and time for PRRSV vaccination as well as the respective schedule

References
Preliminary observations on the genetic heterogeneity of *Theileria equi* and *Babesia caballi* in the horse population of central Italy

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*T. equi* and *B. caballi* are distributed worldwide, except for Australia. Phylogenetic analysis was conducted on sequences of 78 positive blood samples collected in 2013/14, having either low Ct values in real-time PCRs (46 samples) for the parasites (1, 2) or PCR pos/ELISA neg (VMRD, USA) results (32 samples), from symptomatic and asymptomatic horses of central Italy. Case definition for the acute form of piroplasmosis was temperature >38°C and at least one of the following signs, jaundice, anemia and petechial hemorrhages, and a PCR positive (+) result. Sequencing was performed on the V4 hypervariable region of the 18S rRNA gene (390 bp for *B. caballi* and 430 for *T. equi*). Using BLAST, sequences were aligned with those deposited in GenBank for both piroplasms, having a minimum 98% query coverage and > 97% homology.

Genetic distance and homology confirmed that sequences of both parasites could be divided into the 3 known groups (homology groups>97%). Group 1 included sequences homologous to first-ever reported piroplasms, group 2 to the “like genotypes”1, and group 3 included those with equidistant homology for the two groups (2). Among the 72 sequences for *T. equi*, 39 belonged to group 1, 24 to group 2, and 9 to group 3. Group 1 included 62% (24/39) of the sequences of symptomatic horses, while in group 2 96% (23/24) were from asymptomatic horses. Studies report that sequences within group 1 and 2 are in all endemic areas, while group 1 and 3 only in America. Group 3 was never submitted for Europe and Asia. Of the 27 horses, *T. equi* PCRpos/ELISAneg, 23 (85.1%) had group 1 sequences with 19 of them symptomatic. For *B. caballi*, 7 sequences were obtained, of which 2 were positive for ELISA, 4 belong to group 1, 1 to group 2, and 2 to group 3. No clinical significance was attributed to this parasite due to the limited number of sequences available. Sequences from group 1 and 3 are not reported for Europe and America, while group 2 is in all endemic areas. Sequences within the same groups and their wide distribution suggest that the diversity could be independent from their geographical origin and probably linked with the international movement of equids. **The present study is the first to report group 3 for both parasites in Europe.** Further studies are required to investigate the relatively low sensitivity of the ELISA and the possible correlation of the clinical evolution of the infection with the genetic group. On the basis of the above results, possible correlations between genetic grouping, clinical status and serological reactivity need to be further investigated.

**References**

Bovine tuberculosis (TB), mainly caused by Mycobacterium bovis, is a zoonotic disease with implications for public health and having an economic impact due to decreased production and limitations to trade. The tuberculin skin test is the prescribed primary test for trade of cattle. The in vitro gamma-interferon (IFN-γ) assay (BOVIGAM®, Thermo Fisher Scientific) was developed in Australia in the late 1980s (1) and had been recommended by the OIE since 1996 (OIE Terrestrial Manual) as a laboratory-based test ancillary to the skin test. Most TB control programs rely on the use of BOVIGAM® either as a parallel or serial test to the skin test to confirm or negate an initial reactor in the tuberculin skin test. The assay is approved for use in the European Union since 2002 (Council Directive 64/432/EEC, amended by (EC) 1226/2002), received approval by the USDA in 2003, and has since then received official licensure in many countries worldwide.

The BOVIGAM® diagnostic kit is now proposed for inclusion in the OIE register of diagnostic kits for cattle, goat, buffalo (Syncerus caffer) and sheep certified by the OIE as validated as fit for the following purposes:

• Eradication of TB infection from defined populations
• Re-establishment of freedom after TB outbreak situations
• Certify freedom from TB infection or agent in individual animals or products for trade/movement purposes
• Historical freedom of TB
• Confirmatory diagnosis of suspect or clinical TB cases (includes confirmation of positive screening tests)
• Estimate prevalence of TB infection to facilitate risk analysis (surveys/herds health schemes/disease control) and,
• Ancillary test for eradication of TB

Reference
Paratuberculosis (Johne’s disease) is a contagious disease caused by *Mycobacterium avium* ssp *paratuberculosis* (MAP). Although this mycobacterium has been identified in several species, wild and domestic ruminants are the species mainly affected by the disease. The disease is characterized by a progressive inflammation of the small intestine resulting in chronic diarrhea, slow wasting, reduction of fertility and milk production.

The diagnosis of MAP infections is based on the detection of MAP by culture or PCR and/or the demonstration of an immunologic response. The most popular immunologic assays detect anti-MAP antibodies in blood or milk. Among the assays, the most sensitive are ELISAs. Most commercial ELISAs use as antigen an aqueous extract of MAP. They also include an absorption step for the samples using an environmental mycobacterium, *Mycobacterium phlei*.

The diagnostic sensitivity of the MAP ELISA varies greatly depending on the stage of the disease. In the early stages, the test sensitivity is very low (e.g., <30%). While the disease is progressing, the test sensitivity also increases and may reach 80%.

Recently, Biovet has developed an ELISA based on a MAP antigen (ethanol extract) and an absorbent different from those used in other assays. The simultaneous use of these components provides original properties to the test. Moreover, the use of antigen-coated and uncoated wells (2-well test) also contributes to the test specificity.

Serum samples from 350 and 150 cattle expected to be free or infected with MAP were examined with the Biovet ELISA, the Idexx MAP ab test (Idexx), and the Parachek test (Life Technologies). **Results suggest that the Biovet assay has diagnostic sensitivity and specificity similar to those of the other 2 commercial kits.** Interestingly, some samples from infected cattle were positive in only one of the tests.

**References**
The purpose of this paper is to compare the serology of the infectious bursal virus using 4 commercially available kits (CIVTEST AVI IBD, ELISA-I, ELISA-S, and ELISA-B), all of which are indirect ELISAs. To do so, broilers were selected from 6 different farms of France. All groups of animals were vaccinated with the live, attenuated vaccine, HIPRAGUMMI BOREO G97. A representative sample of sera from each of the 6 groups was analyzed when they were 1-day-old, on the day of vaccination, and at the time of euthanasia.

All of the kits detected maternal immunity, loss of passive immunity, and seroconversion due to vaccination, except in one batch of animals in which maternal antibodies neutralized the vaccination. As an example of titer values at 1 day of age, the mean values obtained in the various groups of animals were with CIVTEST AVI IBD, ELISA-I, ELISA-S and ELISA-B, respectively, as follows: 5805, 4505, 4566, 5787; the coefficients of variation were: 26%, 28%, 32%, and 34%. In almost every situation, the ELISA-B kit was that which presented higher results. On the day of vaccination, the ELISA-S kit showed the greatest difference in results in terms of coefficient of variation due to the presence of a high number of 0 values.

At 1-day of age and at the time of euthanasia, the 4 ELISAs exhibited similar kinetics and offered the same interpretation of results in the 6 batches of animals. The greatest differences are observed on the day of vaccination.
Application of multilocus variable-number tandem-repeat analysis for typing \textit{Salmonella} Enteritidis isolates from poultry-related sources in Canada

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\textit{Salmonella enterica} subsp. \textit{enterica} serovar Enteritidis (\textit{S. Enteritidis}) is a pathogen of public health and food safety concern as it is the most prevalent serotype isolated from humans diagnosed with salmonellosis and is largely associated with poultry and poultry products. The presently used subtyping methods, such as phage typing and pulsed-field gel electrophoresis (PFGE) provide insufficient \textit{S. Enteritidis} discrimination for epidemiologic and source attribution investigations. In this study, we evaluated a multiple-locus variable-number tandem-repeat analysis (MLVA) scheme for subtyping of \textit{S. Enteritidis} isolates that originated from poultry-related sources in Canada.

A PulseNet International MLVA typing scheme targeting 7 variable-number-tandem repeat (VNTR) loci was evaluated on 249 \textit{S. Enteritidis} isolates collected from poultry environments, hatcheries, and food products over the period 2001-2013. Discriminatory indexes for each MLVA locus were assessed on a subset of 91 epidemiologically unrelated \textit{S. Enteritidis} isolates. MLVA locus VNTR05 provided the most discrimination with 14 different alleles and a Simpson’s diversity index of 71.7; diversity indexes for other loci varied between 0 and 44.5. The VNTR loci demonstrated 100\% in vitro stability; MLVA showed 99.5\% and 98.6 \% intra- and inter-laboratory reproducibility, respectively. Discriminative properties of PFGE and MLVA typing approaches were compared for a subset of 91 epidemiologically unrelated \textit{S. Enteritidis} isolates. Overall, 35 MLVA types (index of discriminatory power DI=0.8278) were identified for this set of isolates represented by 13 phage types, while only 14 PFGE profiles (DI=0.7375) were observed. A combination of PFGE and MLVA typing approaches generated 41 PFGE/MLVA types (DI=0.9158). The MLVA approach considerably improved typing of \textit{S. Enteritidis} isolates with a phage type PT8 and PFGE type SENXAI.0003-SENBNI.0003 (41.8\% of \textit{S. Enteritidis} isolates in our study), and further discriminated this group into 11 MLVA types. The MLVA did not improve subtyping of another highly clonal \textit{S. Enteritidis} group with a phage type PT13a and PFGE pattern SENXAI.0006-SENBNI.0007 (13.2\% of \textit{S. Enteritidis} isolates in the study), as 85\% of these isolates belonged to the same MLVA type.

Overall, MLVA for \textit{S. Enteritidis} demonstrated excellent intra- and inter-laboratory reproducibility, was more discriminatory, and cost and time efficient when compared with the ‘gold standard’ PFGE. The MLVA results presented in numerical strings could be easily exchanged and evaluated between laboratories. The method can provide valuable additional information and can be adopted for routine \textit{S. Enteritidis} subtyping in diagnostic microbiology laboratories.
067 Survey indicates circulation of 793/B and QX-type infectious bronchitis viruses in Hungary in 2014

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Understanding the epidemiology and improving vaccinal protection against the highly variable chicken Infectious bronchitis virus (IBV) requires the knowledge of the circulating serotypes/genotypes of IBVs on defined geographic areas. Accordingly, we initiated a survey among the major poultry producers in Hungary in order to reveal the prevailing IBV serotypes.

Tracheal swabs and organ samples (cecal tonsils, kidneys, trachea) were collected from broiler, layer, and meat-type breeder flocks, and were subjected to IBV detection by virus isolation and polymerase chain reaction (PCR). The IBV positive samples were further characterized by nucleotide sequencing/phylogenetic analysis of a portion of the S1 IBV gene.

Seventeen submissions proved to be positive for IBV: 10 4/91, 6 QX, and 1 D274 serotype positive submissions were found. One sample contained a mixture of QX and Massachusetts serotype viruses. Most of the detected 4/91 and the D274 viruses presumably represent vaccine strains.

The proportion of the QX positive samples and their observed variation coincides with the situation in a few European countries (1).

The detected viruses clustered geographically, although homology was revealed even between strains detected from geographically distant places, suggesting epidemiologic link(s) between these farms. Noticeably, the Imrehegy origin QX-like strain, collected in 2011, separated from the rest of the QX-like group and showed high homology with A2-type strains from Xindadi and Xinnong (2), regarded as nephropathogenic variants of IBV. Therefore, this strain deserves further analysis.

Updated regularly, the preliminary virus map will be useful both for the adjustment of vaccination protocols and supporting diagnostic labs with significant information.

References
Within-pen prevalence performance of the IDEXX PRRS OF test for detection of antibodies in swine oral fluids

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The IDEXX PRRS OF test (IDEXX Laboratories, Westbrook, ME) is the first commercially available same-day protocol ELISA to detect antibodies to porcine reproductive and respiratory syndrome virus (PRRSV) in swine oral fluids. The sample is obtained by hanging 3-strand cotton rope within reach of the pigs and allowing them to chew on it, thus transferring a composite sample of oral fluids to the rope. In a pen-based sample of oral fluids, the pen represents the infectious unit, and the collected sample is a composite of oral fluid material from those pigs in the pen that chewed on the rope. The probability of detection of PRRSV antibodies in oral fluids collected from a pen of pigs has been previously evaluated as a function of within-pen prevalence, using a non-commercial adaptation of our serum test, PRRS X3 (IDEXX Laboratories) (1, 2).

In this study, we evaluated the performance of the commercial IDEXX PRRS OF test as a function of within-pen prevalence using the samples from this former study. The design of the prevalence study has been described previously (1). Briefly, pigs vaccinated with modified live vaccine (Ingelvac® PRRS MLV) were introduced into pens of pigs seronegative for PRRSV antibodies at different prevalence of vaccinated (seroconverted) pigs (0%, 4%, 12%, 20%, 36%); 5 pens were studied for each prevalence level. Blood samples were drawn from all pigs to confirm serologic status. Oral fluids were collected from each pen by hanging a cotton rope in the pen for 30 min; 5 successive replicate collections were done per pen. Serum was tested with PRRS X3 to verify PRRS serologic status of each pig, while the oral fluid samples were tested using the IDEXX PRRS OF test as well as the published adaptation of PRRS X3 (2). The probability of detecting a positive oral fluid sample was modeled as a function of within-pen prevalence using a logistic regression model on SAS, with both the pen and the rope sample within a pen considered as random effects. Results indicated that among the 100 oral fluid samples from those pens containing at least one seropositive pig starting at a prevalence level of 4% up to 36%, 73% were positive by the IDEXX PRRS OF test compared to 58% using the standard overnight procedure. The detection probability with the PRRS OF test was 91% at a prevalence of 20% and 99% at a prevalence of 32% or higher.

The enhanced sensitivity of the commercial product is designed to support the use of pen-based oral fluid sampling for PRRS surveillance in commercial pig populations.

References
Enteric infection is one of the frequent diseases that are responsible for significant economic losses caused by reducing rate of weight gain and increasing mortality in swine. It has mainly been associated with enteric bacteria in grower/finisher pigs. For instance, *Lawsonia (L.) intracellularis*, *Salmonella (S.)* serovar Typhimurium and *Brachyspira (B.) hyodysenteriae* are known as the agents of porcine proliferative enteropathy (PPE), swine salmonellosis (SS), and swine dysentery (SD). Therefore, the aims of this study was to investigate the frequency of enteric bacteria including *L. intracellularis*, *S. Typhimurium* and *Brachyspira* spp. in the diarrheic finisher pigs and to differentiate the *Brachyspira* spp.

From March to December 2014, 631 feces of diarrheic pigs were collected to investigate the infection of *Brachyspira* spp., *L. intracellularis*, *E. coli*, and *Salmonella Typhimurium* from 41 farms all over the country. Samples were smeared on blood (ASAN, Korea) and MacConkey (BD, USA) agar for *E. coli*, modified CVS agar for *Brachyspira* spp.. In addition, Rappaport-Vassiliadis broth, CHROMagar Salmonella Plus (CHROMagar, France), and XLT4 agar (Becton, USA) agar were used for culture of *Salmonella* spp. Plates were incubated aerobically at 37°C for 2 days and under anaerobic conditions at 37°C for 5 days. Conventional PCR was performed to identify *Brachyspira* spp., *Salmonella* spp. and *L. intracellularis*. Real-time PCR were applied to differentiate *Brachyspira* spp.

Among 41 herds and 640 samples, the incidence of *L. intracellularis*, *Brachyspira* spp., and *Salmonella* spp. were 24.4%, 31.7% and 7.3% in herds, and 3.3%, 5.0% and 1.5% in pigs, respectively. Of *Brachyspira* spp. positive samples, *B. murdochii* (46.2%) predominated followed by *B. hyodysenteriae* (27.0%), *B. pilosicoli* (15.4%), *B. intermedia* (11.5%), and *B. innocens* (3.8%), and one isolate was contained both *B. murdochii* and *B. intermedia*.

In Korea, *Brachyspira* spp. was the most frequent pathogen, followed by *L. intracellularis* and *Salmonella Typhimurium* in diarrheic grower and finisher pigs. *B. hyodysenteriae* has been mainly focused on in Korea but, this study showed that *B. murdochii*, *B. pilosicoli*, *B. intermedia*, and *B. innocens* were also widely detected in diarrheic grower and finisher pigs. Moreover, *B. murdochii* was isolated much more than *B. hyodysenteriae*. These data may contribute to understanding the distribution of frequency of enteric bacteria in diarrheic grower and finisher pigs.
Porcine pleuropneumonia is a dreaded disease that causes respiratory problems and mortality in piglets and fattening animals mainly caused by *Actinobacillus pleuropneumoniae* (APP). To date, there are 15 recognized serotypes designated 1 to 15, which differ greatly in their virulence potential. Diagnostic tests are directed either to a small number of specific highly virulent serotypes, or to cover all serotypes. In the immunological tests, there are several antigens that are targeted as diagnostic tools for APP serotypes, the most important are toxins ApxI, ApxII, and ApxIII. Some of APP serotypes express a single Apx toxin gene, while others have a combined expression of both, providing a greater cytotoxic potential. Another toxin, ApxIVA, is present in all serotypes of APP during in vivo infection and leads to highly species-specific antibodies. However, these methods are time-consuming and require specialized equipment operated by trained technicians. A new rapid immunochromatographic strip assay (ICSA) was developed. The purpose of this study was to evaluate a simple strip assay (based on a chromatographic and immunogold system) for rapid in situ detection of APP antibody in swine sera.

This ICSA uses the S4070 strain of serotype 1 APP from which a truncated ApxIVA gene (ApxIVtr) of 1.5 kb is amplified by PCR so as to contain potential antigenic sites recognized by clinical sera. An *E. coli*-expressed recombinant truncated ApxIV protein antigen of 56 kDa was used as the capture protein for detecting antibodies against APP. In this study, the performance of this assay was evaluated with sera from both clinical samples and experimentally infected piglets. Detection by immunochromatographic strip assay was compared with detection by a standard, commercially available, indirect enzyme-linked immunosorbent assay and western blot assays. The ICSA detected antibodies in sera known to contain antibodies to APP in clinical samples. The sensitivity and specificity of this test were evaluated.

These tests were found more suitable for rapid diagnosis of APP infection and could be used as a first-line detection system for screening of pig infected under field conditions.
071 Metabolomic profile of broiler chickens following administration of oligodeoxynucleotides containing CpG motifs (CpG-ODN)

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The complete set of small molecules found within a biological sample, organ tissue or cell, is called the “metabolome”. Metabolomic analysis assists in understanding the changes occurring in the metabolome due to disease, medication, immunization, or stress. We have previously shown that oligodeoxynucleotides containing CpG motifs (CpG-ODN) protect broiler chickens against *Escherichia coli* septicemia. In an attempt to discover the mechanism behind this immune protection, we designed a study to identify the metabolomic profile of broiler chickens following administration of CpG-ODN. In this experiment, 11-day-old birds were divided into 1 groups; 1 group was administered CpG-ODN intramuscularly (50 μg/50μL/bird) and the control group was administered saline (50 μL). Blood was collected from 5 birds per group at 3 and 24 hours post-administration of CpG-ODN or saline. Serum was separated and metabolite profiles were analyzed using nuclear magnetic resonance (NMR) spectroscopy and direct injection liquid chromatography mass spectrometry (DI/LC-MS/MS) techniques. Analysis of metabolomic data was conducted using MetaboAnalyst 2.5 software.

We observed that the serum metabolome differed between CpG-ODN-treated and saline-treated groups for a variety of metabolites including acetone, myoinositol, 3-hydroxybutyrate, glutamine, phenylalanine, malonate, alpha-aminoadipic acid, several glycerophospholipids, and sphingolipids. We believe that the metabolomic profile will further help our understanding of biochemical pathways associated with CpG-ODN mediated immunomodulation.

**Funding Agencies**: NSERC, Alberta Livestock and Meat Agency (ALMA), Chicken Farmers of Saskatchewan, Canadian Poultry Research Council, Alberta Chicken Producers.

*Graduate student presenter
072 Sensitivity evaluation of the ID Screen® paratuberculosis indirect ELISA in ovine sera

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Paratuberculosis diagnostic methods include the detection of antibodies in serum or milk by ELISA, or direct detection by PCR or fecal culture. The aim of this study was to evaluate the sensitivity of the ID Screen® Paratuberculosis Indirect ELISA in ovine sera.

Test specificity, evaluated on disease-free ovine herds, was found to be greater than 99%. Test sensitivity was evaluated on experimentally-infected animals. Six dairy ewes between 3 and 9 years of age were experimentally-infected with an ovine MAP strain. Animals were bled every 2 weeks up to 290 days post-infection (dpi). Antibody levels increased as of 290 dpi. In a separate study, 9, 10- to 11-month-old lambs from serologically-negative sheep were studied. Five of these lambs were experimentally-infected with an ovine MAP strain; the other 4 lambs were experimentally-infected with 2 bovine MAP strains. Animals were bled every 2 weeks up to 199 dpi (ovine strain) or up to 290 dpi (bovine strain). Antibody levels increased between 116 and 240 dpi.

321 sheep sera from 39 naturally-infected flocks were tested. All 321 animals were analyzed by both the ID Screen® ELISA and by morphologic pathology. ELISA and pathology test agreement was 81.93%.

In conclusion, seroconversion in sheep was clearly observed as of 209 dpi, whereas seroconversion in lambs was strain-dependent, appearing earlier in lambs infected with an ovine strain compared with sheep infected with an ovine strain, or lambs infected with a bovine strain. One of the 4 lambs infected with a bovine strain seroconverted as of 240 dpi. High variability between animals was observed, especially for lambs.

References
1. Manual of Recommended Diagnostic Techniques and Requirements volume III – Paratuberculosis 5B/009.
Swine influenza viruses (SIV) are a major cause of respiratory disease in pigs worldwide, causing considerable morbidity, but low mortality. Fast mutation in hemagglutinin genes causes continuous change on surface glycoproteins of influenza viruses resulting in immune escape from the neutralizing antibody response. Since 1998, 3 predominant subtypes of influenza viruses (H1N1, H1N2, and H3N2) have been circulating in the Canadian swine population. The objectives of this study were to genetically characterize the SIV strains that currently circulate in Quebec swine herds.

In order to meet this objective, 30 SIV PCR-positive samples from swine with clinical problems due to respiratory disease were isolated from Quebec commercial farms. All isolates were subtyped and the molecular evolution of all gene segments was analyzed.

Lung tissue, 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 8 genes of the viral genome were individually amplified by RT-PCR and were subsequently sequenced.

To date, only 10 viruses have been isolated and analyzed. Data analyses revealed the presence of 2 main genotypes, H1N1 and H3N2. The hemagglutinin gene analyses indicated that 6 viruses are similar to the SIV North American triple-reassortant (sw-H3N2) allowing the classification of these viruses into the swine H3 cluster IV. These results of whole-genome analysis demonstrated that multiple genetic reassortments occurred between sw-H3N2 and pH1N1, and resulted in the appearance of 5 new profiles. The 4 remaining SIV strains were found to belong to sub-type H1N1swH1γ group, while analysis of the whole-genome revealed 2 different profiles resulting from a genetic reassortment between sw-H3N2 triple-reassortant and pH1N1.

Furthermore, amino acid substitutions were detected on the antigenic sites of the hemagglutinin. Antigenic evaluation, using specific swine sera is in progress.

The results suggest that swine influenza virus continues to evolve in Quebec swine herds and that surveillance should be maintained to detect important genetic changes that could undermine the diagnostic test and vaccine efficiencies.

*Graduate student presenter
Seroprevalence of bovine brucellosis in slaughterhouses with inspection service in the state of São Paulo, Brazil

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This study estimated the prevalence of bovine brucellosis in the state of São Paulo as well as the prevalence in slaughterhouses with different levels of sanitary inspection in the same state after the implementation of the National Program for the Control and Eradication of Brucellosis and Tuberculosis (PNCEBT) established in 2001.

We sampled 1,490 animals in 9 slaughterhouses distributed in different regions of the state, 3 with federal inspection (FI), 3 with state inspection (SI), and 3 with municipal inspection (MI). Sera were analyzed by the rose bengal test (RBT) and the complement fixation test (CFT). The prevalence obtained through CFT in different inspection services were, 0.40% [0.00% - 0.95%] (FI), 2.00% [0.77% - 3.23%] (SI), 4.29% [2.67% - 6.48%] IC95% (MI) and the state prevalence was estimated at 2.21% [1.47% - 2.96%]. The multivariate logistic regression analysis revealed the odds ratio of find a brucellosis animal. Statistical difference was observed among the 3 levels of sanitary inspection (p<0.0015), where an inverse relationship was noted between the hierarchical level of sanitary inspection with the possibility of find positive animals. Slaughterhouses with municipal inspection showed 11.5 times more likely to have brucellosis animals in comparison with FI, and 2.3 times more likely than state slaughterhouses. Slaughterhouses with SI were almost 5 times more likely to cull animals with the disease in comparison with FI. With the results obtained, it was possible to evaluate the situation of bovine brucellosis in the state of São Paulo as well as the difference between the current inspection systems in the country, demonstrating a decrease in surveillance criteria according to the hierarchical level.

We concluded that the seroprevalence of bovine brucellosis in the state of São Paulo remained stable, and the adoption of new measures to enhance the PNCEBT could accelerate the control and eventual eradication of the disease. In addition, we conclude that increased surveillance in slaughterhouses with state inspection and municipal inspection could help in disease control.
075 Enzootic bovine leukosis: serologic variation in the peripartum period

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Factors such as stress, physiologic status, and pregnancy may interfere with the serologic diagnosis of diseases because of the variation of immunoglobulin levels in the bloodstream. This may make difficult the control of diseases due to the impossibility of detection of positive animals and thus the elimination of these sources of infection. This study demonstrates the influence of pregnancy in serum diagnosis of enzootic bovine leukosis (EBL) and emphasizes the importance of routine testing for maintaining the health of livestock.

Considering this, 143 pregnant females were sampled 30 days before and 15 days after calving. Samples were submitted to agar gel immunodiffusion (AGID) testing for enzootic bovine leukosis diagnosis. In 76 serum samples (53.15%), different AGID results were observed before and after calving.

It is clear that the levels of various serum immunoglobulins vary in blood peripartum. Therefore, the use of a single sample for serologic testing may not accurately represent the serum antibody concentration without knowing the physiologic and reproductive status of the individuals tested.

The results indicate the importance of conducting periodic tests for the correct detection of animals positive for the disease.
076 Profile of cytokines and chemokines triggered by wild-type strains of rabies virus in mice


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Rabies is a lethal infectious disease that causes 55,000 human deaths per year, transmitted by different mammal species, as dogs and bats. The host immune response is essential to avoiding viral progression and promoting viral clearance. Cytokines and chemokines are crucial in the development of immediate antiviral activity and, in order to succeed, the rabies virus has to evade this immune response. The virus’ capacity for evasion is correlated with rabies virus pathogenicity and inflammatory response level with the most highly pathogenic strains being the most efficient in hijacking the host’s defense mechanisms and thus inflammation.

The purpose of this study was to evaluate the gene expression through real-time RT-PCR of a set of cytokine and chemokine genes related to immune response, such as CCL2, OAS1, IL2, IL6, IL12, TNFα, IFN γ, IFNβ, CXCL10, CD200R and IGF1 in brains of mice inoculated intramuscularly (IM) or intracerebrally (IC) with different lethal doses of 2 wild strains of rabies virus, one from dog (V2) and another from vampire bats (V3). Animals inoculated IM were euthanized at 5 and 10 days post-inoculation (dpi) when whole brain was removed; in groups inoculated IC, brains were removed immediately after natural death. Relative gene expression of cytokines and chemokines in 80 DL50V2 group showed IFNβ highly expressed at 5 dpi (p<0.001); at 10 dpi IL12 had the higher expression (p<0.01); in the comparison between 5 and 10 dpi CD200R (p=0.001), IL12 (p=0.01), and IFNβ (p=0.06) had different expression. At 40 DL50V2 group; IL12 (p<0.05) had higher expression at 5 dpi; OAS1 (p=0.01) and IFNβ (p=0.01) were higher at 10 dpi; comparing 5 dpi versus 10 dpi IL12 (p=0.09) showed a slight increase. At 40 DL50V3 group; IFNβ (p<0.01) and CCL2 (p<0.001) had a higher expression at 5 dpi. No difference was observed at 10 dpi, although in the comparison between 5 dpi versus 10 dpi TNFα (p=0.0005), IFNβ (p=0.0002), IL12 (p=0.0047) and CD200R (p=0.0047) showed a higher expression at 10 dpi. In animals inoculated IC, both variants showed an increase in the expression of IFNγ (p<0.001), CXCL10 (p<0.001), TNFα (p<0.05) and CCL2 (p<0.001).

The results demonstrate that the gene expression profile is intrinsic to the specific rabies variant. More efficient virus evasion, related to IFNβ inhibition, was observed in the group infected with a high lethal dose. The precocious production of cytokines and chemokines seems to be more important than their levels of expression for rabies survival.
077 First isolation of canine parvovirus in Morocco

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Canine parvovirus 2 (CPV-2) is an acute infectious and highly contagious virus which has evolved since its emergence in the mid-1970s, giving rise to new antigenic variants (CPV-2a, CPV-2b, CPV-2c) with increased pathogenicity and extended host ranges. CPVs are considered to be the most dreaded enteric pathogen of canine population worldwide, in spite of widespread vaccination of domestic dogs.

Although clinically suspected in Morocco since 1984, first evidence of CPV circulation was confirmed in 2011 (1), even in dogs ‘regularly’ vaccinated. Further studies typed autochthonous strains as the original strain CPV-2 and the new antigenic variant CPV-2c (2). The present study was conducted to isolate autochthonous CPV in cell culture (Madin-Darby canine kidney MDCK) from suspect lethargic dogs suffering from vomiting, and bloody diarrhea.

Eighty-three clinical specimens (rectal swab, gut content, colon, liver, spleen, myocardium) were collected from 6 “regularly” vaccinated and 23 unvaccinated dogs. As an initial step, the panel was screened with the hemagglutination (HA) test. Virus isolation was carried out on samples that had tested highly HA-positive. After 3 passages, viral replication was confirmed first by HA and second by real-time PCR assay which has become the principal rapid, specific, and sensitive tool for detection of CPV strain DNAs.

The screening HA test was able to detect CPV antigens in 24/83 analyzed samples collected from 18 symptomatic dogs. According to the results of HA and rt-PCR assays, cultivation of canine parvovirus in MDCK cell lines was successfully established from 11 specimens collected from 10 symptomatic dogs. Additional passages of cell cultures were done in order to obtain a high yield stock of viruses for subsequent assays. **To our knowledge, this study reports the first isolation of canine parvovirus in Morocco.**

References
Validation of the ID Screen® African swine fever indirect ELISA

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ASF control and eradication programs require accurate and reliable diagnostic tests. The ID Screen® African Swine Fever Indirect ELISA detects anti-ASFV antibodies in both domestic and wild pigs. Unique features of the ELISA include the coating of 3 recombinant ASFV antigens (P32, P62, and P72), and the ability to use the test with blood filter paper and meat juice as well as serum and plasma. This study presents validation data obtained for this test.

Specificity was evaluated through the analysis of 763 disease-free sera from domestic pigs, wild boars, and Iberian pigs. Measured specificity was 99.61% (CI 95%: 98.96% - 99.90%).

Measured specificity for 100 samples from disease-free animals from France was 100% (CI 95%: 96.30% - 100%). 90 negative sera were also tested in parallel by both the serum and filter paper protocols. All 90 samples were correctly identified as negative by both protocols.

Test sensitivity was evaluated through the analysis of 3 sera from vaccinated and challenged pigs. These sera gave positive results with the ID Screen® ELISA. 8 reference sera from the ASF European Reference Laboratory were also correctly identified as positive.

3 positive sera were titrated and tested by both the serum and filter paper protocols. The measured analytical sensitivity was similar regardless of the sample type tested.

Test sensitivity for meat juice was evaluated through the analysis of spiked samples, which were all correctly identified as positive.

In conclusion, the ID Screen® African Swine Fever Indirect ELISA is the only commercial ELISA based on the use of 3 different recombinant proteins. A flexible tool, the test may be used for filter paper and meat juice samples. It shows excellent specificity and sensitivity, correctly detecting reference sera from the EURL for ASF (INIA-CISA, Madrid, Spain).

The use of filter papers makes sampling easier, especially for wild boars. By using the elution protocol in deep-well tubes with direct transfer to ELISA plates with a multi-channel pipette, sample identification errors may be avoided.

This test is a reliable tool for the detection of antibodies against the ASFV in both domestic pigs and wild boars. The serum application has been validated by the ASF European Reference Laboratory.
079 Development and validation of a new ASFV ELISA

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The current outbreak of the highly contagious African swine fever virus (ASFV) strains in Eastern Europe is causing high economic losses. ASFV is already endemic in parts of Africa and southern Europe. Without adequate disease measurement and control programs, the disease can spread into other parts of Europe with the potential to become endemic in these regions. For disease monitoring, the direct virus detection by qPCR as well as the indirect detection of the resulting immune response after infection by ELISA is of importance. In order to facilitate this, we have developed an indirect ELISA, the LSIVet™ Porcine African Swine Fever (PPA) – Serum ELISA, which is based on a semi-purified ASFV antigen for the detection of ASFV antibodies in serum.

In order to demonstrate the sensitivity and specificity of the new LSIVet™ ASFV kit, different internal and field studies including animal infection experiments (INIA, Valdeolmos, Spain; CVI, Netherlands; Germany) were carried out. In total, 1,978 negative samples from ASFV-free regions in Germany, Spain, Netherlands, and France were tested to demonstrate specificity of the assay. This testing included 130 samples that were positive for other major porcine diseases. The results showed 99.4% specificity of the ELISA with no detection of any of the samples that were positive for other porcine diseases. For validation of sensitivity, 600 ASFV-positive samples from Africa and Europe were tested. The test results showed 99.5% sensitivity.

In summary, the new LSIVet™ Porcine African Swine Fever (PPA) – Serum ELISA proved to be a reliable, sensitive, and specific tool for detection of ASFV antibodies. The ELISA represents the prerequisite for effective ASFV monitoring and control.
Comparison of heart weights, gross and histological lesions between market hogs that died in transit to the abattoir and hearts from normal market hogs

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The cause of death for hogs in transit is frequently labelled as heat stress. Large numbers of hogs are exposed to the same environmental stress; however in-transit losses (ITL) are low, 0.1% of hogs shipped to market in Canada. The objective of this study was to determine if pre-existing cardiac pathology predisposes hogs to death during transit.

Hearts collected from the processing line (non-ITL hearts, N=167) were examined and compared to ITL hearts (N=142). Comparison of the weights of total heart (TH), left ventricle and septum (LV+S), right ventricle (RV), and body (B) for 167 non-ITL hearts collected from the processing line and 142 ITL hearts were completed. Gross and histological findings were scored from 0-3 representing none, mild, moderate, and severely affected.

ITL versus non-ITL weights were: TH*(456.99 g +/- 75.8 vs 389.68 g +/- 49.7, LV+S*(281.10 g +/- 31.7 vs 247.96 +/- 45.5), RV*(104.98 g +/- 22.4 vs 89.65 g +/- 14.5), RV/LV+S(0.38 +/- 0.06 vs 0.36 +/- 0.05), B(121.69 kg +/- 9.8 vs 122.62 kg +/- 5.8), TH/B*(3.75g/kg +/- 0.52 vs 3.16g/kg +/- 0.46) *indicates value was significantly greater for the ITL group (p<0.05).

ITL hearts had greater frequencies of dilation of the aorta and pulmonary artery (74% vs. 0.6%), atria (46% vs. 0), RV (88% vs. 2%), moderate to marked LV lumen dilation (42% vs. 10%), and thickened atrioventricular valves (25% vs. 2%) over non-ITL hearts (Chi square test p<0.05). Variable severity of LV+S and RV thickening were present in both ITL and non-ITL hearts. ITL hearts had greater frequencies of acute hyaline and/or granular degeneration of myocardial fibers (16% vs. 7%), endocardial fibrosis (27% vs. 14%), and moderate to marked interstitial fibrosis (8% vs. 2%) over non-ITL hearts (Chi square test p<0.05). Medial hyperplasia of the coronary arteries, nuclear rowing, irregular bundles of hypertrophic myocardial fibers, and atrophy and fatty replacement of myocardial fibers were present with variable severity in both ITL and non-ITL hearts.

**ITL hogs have pre-existing lesions that result in acute heart failure on the truck not due to heat stress as previously reported.** Lesions observed in non-ITL hogs may be an earlier stage of the lesions observed in ITL hogs. The gross and histological lesions found in Ontario hogs are similar to those reported as hypertrophic cardiomyopathy (HCM) by Liu et al. (1). **This study indicates the importance of postmortem examination including heart and body weights when investigating in-transit losses or the sudden death of late-stage market hogs on the farm.**

**Reference**

081 Use of percutaneous ultrasound guided cholecystocentesis as a diagnostic tool for *Platynosomum* spp. infections

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*Platynosomum* spp. are cat-specific parasitic liver flukes found in tropical and subtropical countries. In the United States, *Platynosomum* spp. infections are seen primarily in the southeast and Hawaii. With climatic changes, there is the potential for these parasites to increase their geographic range. Typically infestation levels are low, except in some tropical locations, and eggs are intermittently found in the feces, making diagnosis challenging. Other diagnostic methods have included ultrasound, liver biopsy, and cholecystocentesis, but a comparative assessment of these methods has not been performed. Percutaneous ultrasound guided cholecystocentesis (PUC) has been described as a safe procedure in healthy cats but complications have not been investigated in cats with cholecystitis (1). In this preliminary study of 7 cats with high levels of *Platynosomum* infection (81 to >1,000 flukes), fecal egg counts (FECs) using 2 g of feces and double centrifugation with Sheather’s sugar flotation solution (2) were compared to counts from bile (eggs per 10-20 μL) obtained via PUC. Gallbladder (GB) and common bile duct (CBD) pathology and the safety of conducting a PUC were assessed on postmortem.

All fecal and bile samples were positive for *Platynosomum* eggs. However, 2 FECs had unreliable results due to issues with the coverslips; 1 cat had a count >500 eggs per gram of feces (epg). The remaining FECs ranged from 7-189 epg. Counts from bile ranged from 0.8 to 25.8 per μL. Ultrasonographic changes included GB distention, GB wall thickening, dilated bile ducts, tortuous CBD, and changes consistent with periductal inflammation. GBs were sometimes obscured or inflamed, making it difficult to aspirate the entire GB content in 4 cases. Collected bile was very dark and/or turbid. The needle centesis site was evaluated during postmortem examination and no evidence of bile duct leakage was found.

Based on this preliminary data, **bile egg count analysis appears to be an alternative and more sensitive method than fecal analysis to diagnose *Platynosomum* infections, especially when fecal examinations are negative.** PUC can be used to obtain bile, but more cases need to be investigated to more accurately assess the risk of performing PUC from abnormal GBs.

**References**

Anti-diabetic effect of *Withania somnifera* in alloxan-induced diabetic rabbits

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The present work was undertaken to investigate the effects of various extracts of *Withania somnifera* for anti-diabetic activity in alloxan-induced diabetic rabbits. Rabbits were acclimatized for a week to standard laboratory temperature. Animals were fed according to a strict schedule (8 am, 3 pm, 10 pm) with green fodder (*Medicago sativa*) and tap water ad libitum. Animals were divided into 9 groups of 6 rabbits each in a random manner. Body weights and physical activities of all rabbits were recorded before experiments started. The animals of group 1 and 2 were given lactose (250 mg/kg, PO) and *W. somnifera* root powder (100 mg/kg, PO) respectively daily from day 1-20. Animals of group 3 were given alloxan (100 mg/kg, IV) as a single dose on day 1. Powdered root of *W. somnifera* in the doses of 100, 150, 200 mg/kg and its aqueous and ethanol extracts (equivalent to 200 mg/kg of crude drug) were given to the treated animals (groups 4-8), respectively by oral route for three weeks (day 1-20 OD), along with alloxan (100 mg/kg, IV) as a single dose on day 1. Group 9 was treated with metformin (200 mg/kg, PO) daily from day 1-20, along with a single dose of alloxan (100 mg/kg, IV) on day 1.

Fasting serum glucose concentration in groups 3-9 was increased significantly (p<0.05) on day 3, with a maximum increase (215.3 mg/dL) in animals of toxic control (TC) group (3) on day 21 of the experiment as compared to normal control (NC) group (1). Effects of different doses (100, 150, 200 mg/kg, PO) of *W. somnifera* root powder (WS) decreased the fasting serum glucose concentration as compared to toxic control group, with a maximum decrease (88.3 mg/dL) in group 2 (treated control) on day 21 of the experiment. Metformin (200 mg/kg, p.o) (reference control), aqueous extract (AWS) and ethanol extract (EWS) of *W. somnifera* (equivalent to 100 mg/kg *W. somnifera* root, PO) antagonized the effects of alloxan as compared to toxic control group.

These results indicate that *W. somnifera* possess significant anti-diabetic activity.
Comparison of *Staphylococcus pseudintermedius* isolated from urinary and dermatologic infections in dogs

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*Staphylococcus pseudintermedius* is a common colonizer and opportunistic pathogen of dogs; it is the most common cause of pyoderma and otitis externa, and the second most common cause of urinary tract infections (UTIs). Despite the frequency with which *S. pseudintermedius* causes UTIs, the literature describing these infections is sparse, and it is unknown whether these isolates are phenotypically or genotypically distinct from dermatologic isolates. We therefore aimed to compare the antimicrobial susceptibility profiles and presence of exi (a key virulence factor involved in epidermal pathogenesis) of clinical urinary and dermatological *S. pseudintermedius* isolates from dogs.

A collection of 52 canine *S. pseudintermedius* isolates including 15 from dermatologic and 37 from urinary tract infections were obtained from Prairie Diagnostic Services in Saskatoon, Canada. Antimicrobial minimum inhibitory concentrations were determined using the Sensititre system for a broad panel of antimicrobials including: penicillin, ampicillin, oxacillin (with 2% NaCl), erythromycin, clindamycin, tetracycline, tigecycline, trimethoprim + sulfamethoxazole, ciprofloxacin, levofloxacin, moxifloxacin, gentamicin, chloramphenicol, rifampin, vancomycin, linezolid, daptoymycin and quinupristin + dalfopristin. Methicillin (oxacillin) resistance was confirmed by PCR amplification of the *mecA* gene. Primers targeting exi were designed for this study using a previously deposited *S. pseudintermedius* whole genome sequence as a template (GenBank accession number: CP002478). Fisher's exact test was used to compare the presence or absence of categorical traits (resistant vs. susceptible, or presence vs. absence of exi) between dermatologic and urinary isolates.

Chloramphenicol and oxacillin resistance (MRSP) were significantly more common among dermatologic than urinary isolates (P=0.02 for both). Significant differences for other drugs were not identified, although the higher frequency of clindamycin resistance among dermatologic isolates approached significance (P = 0.07). A nearly significant relationship (P=0.055) between the presence of exi in dermatologic (87% of isolates) vs. urinary (57% of isolates) was identified, suggesting that while *S. pseudintermedius* may be an opportunistic pathogen, pathotypes with specific tissue tropisms may exist. The next step in our investigation is to characterize our isolate collection using DNA fingerprinting techniques (e.g., pulsed-field gel-electrophoresis) to determine if urinary and dermatologic isolates comprise distinct lineages of *S. pseudintermedius*. We are continuing to collect clinical isolates to increase our sample size, which was an important limitation of the current study.

*Graduate student presenter*
084 O-serogroup and virulence genes of *Escherichia coli* from the pigs suffering from pre- and post-weaning diarrhea

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Diarrhea caused by pathogenic *E. coli* is one of the ongoing problems in pig industry. Especially, enterotoxigenic *E. coli* (ETEC) and shiga-toxin producing *E. coli* (STEC) are mostly associated with pre- and post-weaning diarrhea (PWD) and edema disease (ED). They produce virulence factors related with adhesion and toxins. The aims of this study were to determine the prevalence of virulence genes and O serogroup of *E. coli* from pre- and post-weaning diarrhea.

From 2013 to 2014, 260 *E. coli* were isolated from diarrheic piglets in 230 farms all over the country. The *E. coli* isolated was identified using the VITEK 2 system (biomeriuex, France). O serogroup was determined by slide agglutination in the OIE *E. coli* laboratory (Université de Montréal, Canada). Virulence genes were determined by PCR as described previously.

Of 260 isolates, 101 (38.8%) could be serotyped. The percentage of serotyped isolates from pre-weaning diarrhea (50.8%) was higher than that (29.2%) from post-weaning diarrhea. O149 (n = 32) was the predominant serogroup followed by O8 (n = 6), O157 (n = 6), and O60 (n = 4). Of virulence factors, EAST-1 (43.5%) was predominant, followed by LT (34.6%), STb (33.5%), and Paa (32.3%). F4 ETEC were highly associated with diarrhea in pre-weaning diarrhea. On the other hand, F4 ETEC, F18 ETEC, F18 ETEC/STEC, and F18 STEC were frequently detected in post-weaning piglets. Diffuse adherence factor, AIDA-I, was closely associated with post-weaning diarrhea but there were no differences in Paa in both age groups. Attaching and effacing gene (*Eae*) was only detected in 4 isolates from post-weaning piglets.

This study showed that O149 was the predominant serogroup in pre- and post-weaning diarrhea. Although F4 ETEC is frequently detected in PWD, F18 ETEC and STEC were also associated with PWD. These data may contribute to understanding the distribution of the virulence genes and serotypes of pathogenic *E. coli* associated with pre- and post-weaning diarrhea in piglets.
Development of a real-time PCR for differentiation of *Brachyspira* spp. in pigs

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Swine dysentery (SD) caused by *Brachyspira* (*B.*) *hyodysenteriae* is a severe mucohemorrhagic enteric disorder causing mortality, lowered weight gain, and economic loss. Although, *B. hyodysenteriae* is the main problem, differential diagnosis should be done because there are several species including *B. pilosicoli, B. murdochii,* and *B. intermedia,* which are related to colitis. The purpose of this study was to develop a real-time PCR to differentiate the *Brachyspira* spp. in pig feces.

Primers were designed by alignment of the sequences (*nox* gene) described by Song and Hampson (2009). Probes (*hyodysenteriae, pilosicoli, intermedia,* and *murdochii*) were newly designed to differentiate the *Brachyspira* spp. Reference strains (*B. hyodysenteriae* B1(ATCC 27164), *B. pilosicoli* P43/3/78, *B. intermedia* (ATCC 51140), *B. murdochii*) from Dr. Hampson (Murdoch University, Australia) and 30 field isolates from diarrheic finisher pigs were used to evaluate the real-time PCR. Template DNA was extracted by boiling. Twenty µL of reaction volume were composed of 1 µL of template DNA, 10 µL of 2 × ExTaq (TAKARA, Japan), 1 µL of the primer mix (10 µM), 1 µL of each probe. Amplification and detection were performed by Rotor-gene 3000 (Corbette Research, Australia). Reaction profiles were composed of an initial cycle of 95ºC for 2 min, followed by 40 cycles of 95 ºC, 30 s and 60 ºC, 60 s. All of the PCR products were sequenced to confirm the amplification of *Brachyspira* spp.

In the reference strains, specific and common probes produced an exponential plot in fluorescence only when the corresponding type of the strain was used as a template in the PCR assay. All of the 30 isolates expressing were determined as *B. hyodysenteriae* (23), *B. pilosicoli* (2), *B. intermedia* (1), and *B. murdochii* (4). The sequencing data corresponded to those of each reference strain.

This real-time PCR assay produced specific amplification to corresponding specific genes and differentiated *Brachyspira* spp. The 30 isolates were determined to *B. hyodysenteriae* (23/30), *B. pilosicoli* (2/30), *B. intermedia* (1/30), and *B. murdochii* (4/30). Real-time PCR can be a useful tool to differentiate *Brachyspira* spp. in one reaction without an additional step.
Equine arteritis virus (EAV) is the causative agent of equine viral arteritis (EVA) and one of the major viral pathogens of horses (1). EAV is genus *Arterivirus* in the *Arteriviridae* family, order Nidovirales. EVA is a respiratory and reproductive disease of horses that occurs worldwide. The vast majority of EAV infections are subclinical, but acutely infected animals may develop a wide range of clinical signs including pyrexia, depression, edema, conjunctivitis, and respiratory distress. The direct consequences of EVA outbreaks are financial losses mainly due to abortions of pregnant mares and death of young foals (2). Following primary EAV infection, up to 70% of the stallions will carry the virus in their reproductive tract, sometimes for years, and will shed the virus in their semen.

Several studies have shown that EAV infection has occurred among horses in North and South America, Europe, Australia, Africa, and Asia. Interestingly, EAV infection prevalence in horses varies between countries and horse breeds. In order to determine equine viral arteritis (EVA) prevalence among the 5,000 horses housed in the Vojvodina region of Serbia, 429 sera from non-vaccinated horses were collected. Serologic analysis of equine sera, collected from 2013 and 2014, was performed using virus neutralization test (VNT) as described by the World Organization of Animal Health (OIE) (3). So far, sera of 156 horses from 10 different stud-farms of the Vojvodina region have been tested. The population tested was composed of 86 stallions, 1-26 years of age, and 70 mares 1-23 years of age. The mean age of the population tested was 9.9 years. Our preliminary results indicated that 121 sera were negative (77.60%), 33 were detected positive (21.15%), 2 sera were cytotoxic (1.25%). Among the positive sera, 15 (45.45%) exhibited an antibody titer range from 4 to 16, 10 (30.30%) sera exhibited a titer range from 24 to 96, and 8 (24.24%) sera had a titer above 128. Moreover, **9 out of 10 stud farms that have been included in this survey exhibited positive horses for EAV showing that EAV is circulating in the horse population kept in the Vojvodina region.** So far, only 1 seropositive stallion has been found positive for the presence of the virus in his semen. Viral characterization of this isolate will be investigated.

References
Equine infectious anemia (EIA) is caused by Equine infectious anemia virus (EIAV) belonging to the Retroviridae family, genus Lentivirus, which also includes human immunodeficiency virus (HIV), bovine and feline immunodeficiency viruses (BIV and FIV), and the visna-maedi virus. The clinical form of the disease was described for the first time in France in 1843 but it was only early in the 20th century that the infectious origin of the disease was clearly demonstrated. The clinical signs associated with the infection appear after an incubation period of 1-2 weeks. They are mainly characterized by fever, anemia, edema, and listlessness. Once the horse is infected, several clinical forms may succeed each other. In the acute form, the horse shows serious clinical symptoms that can lead to death; the chronic form is characterized by a recurrence of clinical phases (such as fever, anemia, and listlessness); and there is also an asymptomatic form. Infected equids never eliminate the virus and remain contagious for other equids even when there are no clinical signs. The blood-borne virus is transmitted from one animal to another mainly by biting insects or iatrogenically through contaminated needles or dentistry equipment. Bloodsucking insects – primarily horse flies and stable flies – are mechanical vectors. Although the virus does not replicate within the insect, the infectious virus can remain in its mouthparts for several hours after a bite. Several studies have shown that EIAV infection has occurred among horses in North and South America, France, Germany, Italy, and Romania. So far, no evidence of the presence of EIAV in horses has been reported in Serbia.

In order to determine EIA prevalence among the 5,000 horses of the Vojvodina region of Serbia, 316 horse sera were collected during 2013 and 2014. Serologic analysis was performed using agar gel immunodiffusion (AGID) test as described by the World Organization of Animal Health (OIE) chapter 2.5.6 and by enzyme-linked immunosorbent assay (ELISA). Those sera have been tested with 3 different commercial AGID tests (IDEXX, ID-Vet, VMRD) and 2 different commercial ELISAs (Synbiotics, VMRD). With the 3 AGID kits, 311 (98.4%) among the 316 tested sera were found negative and only 5 (1.6%) sera were found positive for EIA. With ELISA kits, results were slightly different and were dependent of the kit producer. Indeed, Synbiotics ELISA gave the same results as AGID tests, but VMRD ELISA found 295 negative samples, 5 positive samples, and 16 samples were doubtful.

Our study shows for the first time that EIA is present in Serbia and more specifically in the Vojvodina region. Euthanasia and viral characterization of field isolates collected in Serbian horses will be investigated.
Diagnosis of rabbit hemorrhagic disease (RHD) in Mongolia

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Rabbit hemorrhagic disease (RHD) is a highly contagious viral disease of domesticated and wild rabbits, species *Oryctolagus cuniculus*. Transmission of RHDV is extremely rapid by multiple ways, and its infection is indicated by high mortality, nervous and respiratory signs (1).

By OIE recommendation in case of identification for RHD, diagnoses are performed with RT-PCR, ELISA, and HA tests. Since 2009, all those methods were applied for routine laboratory diagnosis of RHD in Mongolia. Identification of RHD was performed on samples collected from found-dead, experimentally infected, and healthy rabbits by OIE recommended assays.

We did not detect a bacterial or parasitic agent from the internal tissues and blood. Grossly, livers and lung were enlarged. The lungs were diffusely edematous with multifocal hemorrhages disseminated over the partial or whole lung surface. The tracheal mucosa was congested. The liver had multifocal hemorrhages of various sizes with signs of coagulative necrosis.

HA titers of experimentally infected rabbits’ tissues were reasonable and detectable (liver 1:40,960, spleen 1:40, lung 1:20). HA titers of found-dead rabbit tissues gave high titers. Specifically, liver HA end-point titers ranged between 1:2,560 and 1:81,920. In the RT-PCR (VP60 gene) test, 6 of 7 samples were positive (liver 3, spleen 2, lung 2) of found-dead rabbits and experimentally infected rabbits. Each of the samples that tested positive and negative by HA was correlatively positive or negative results by RT-PCR.

We conclude that diagnosis of RHD is based on examination of experimental infection, RT-PCR, and HA test. Results of HA and RT-PCR for the diagnosis of pathogenic RHDV infection have no differences in the field or experimental cases. HA and RT-PCR are rapid and routine tests for the laboratory diagnosis of RHD.

References
CCHF is an expanding tick-borne disease due to the outspread of its infected vectors, especially the genus Hyalomma carried by migratory birds from endemic areas (1). CCHF reports are increasing in countries neighboring Italy (2) for which no previous monitoring studies exist. For this, a serosurvey was performed in coastal provinces of 2 Central Italian Regions (Latium and Tuscany) that include arrival/stop-over areas for migratory birds from CCHF endemic regions. The susceptible sentinel species chosen, the sheep, was considered at high risk of infection, grazing on pastures where Hyalomma is present. Semi-sedentary sheep flocks of the study area presented the target population. Disease detection limit was set to 1% and the sample stratified according to the sheep population per province with 540 blood samples examined. As CCHF serologic commercial tests are unavailable for this species, an indirect ELISA was adapted using as antigen, a recombinant nucleoprotein (rNP), expressed in baculovirus, coded from the S gene of CCHF virus (3) and an anti-sheep IgG, monoclonal antibody (Mab) conjugated with horseradish peroxidase (Sigma-Aldrich). The ELISA has wide antigen coverage, as the NP variability is lower than 4%, including the majority of the circulating viral strains (3). Internal controls per run were a blank reaction, a positive (a CCHF Mab), and a negative control. The latter was prepared from a pool of 74 sheep sera, collected from a region were Hyalomma is absent, which when tested individually, gave an optical density (OD) reading within 2 standard deviations (SD) of the median of 30 replicates of the blank control OD. The positive OD cut-off was set above 2 SD of the average negative control OD value, and all study sera examined in a single dilution of 1/100 reacted with an OD within this interval.

The above method is a valid screening test for its safe and simple use, not requiring elevated biosafety measures. The reported results demonstrate that, in the study area, CCHF is below the detection limit of the survey. However, parallel investigations on migratory birds arriving or passing through this region confirm their departure from CCHF endemic areas with risk of introducing the infection. In consideration of this and due to public health relevance of CCHF, the indirect ELISA can be adopted for the continuous monitoring in regions at risk.

References
Portable reverse transcription insulated isothermal polymerase chain reaction (RT-iiPCR) assay for rapid detection of porcine epidemic diarrhea virus

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Porcine epidemic diarrhea (PED) is a highly contagious, acute enteric disease of swine of all ages characterized by diarrhea, vomiting, and dehydration. The clinical manifestation can be mild in adult pigs, but severe in neonatal piglets with mortality rates reaching 100%. The causative agent, PED virus (PEDV), is an alphacoronavirus of the family Coronaviridae. PED was first identified in Europe in 1971, and later spread to Asian countries. In April 2013, the first case of PED was reported in the USA. Since then, this virus has killed millions of pigs and spread to neighboring countries such as Canada, Mexico, the Dominican Republic, Colombia and Peru. There is no treatment for PED, and the rapid application of biosecurity measures is the only method of containment.

Many conventional and real-time RT-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.

Portable, user-friendly, on-site molecular assays can overcome these impediments and facilitate early detection of PEDV. Here we report the development and evaluation of a portable, reverse RT-iiPCR assay for sensitive and specific detection of PEDV. The assay specifically detected PEDV isolates from the USA, Canada, and UK, and it was as sensitive as the real-time RT-PCR assay currently in use at many Canadian animal health laboratories. The assay did not detect transmissible gastroenteritis virus, porcine respiratory coronavirus, or porcine deltacoronavirus, 3 related coronaviruses of swine, and porcine circovirus 2, swine influenza virus, and porcine reproductive and respiratory syndrome virus, 3 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 stored and 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 “positive” or “negative” after the end of a run without the need for user analysis.

Considering the overall speed, ease of performance, versatility, cost and portability, the PEDV RT-iiPCR assay can be used for timely detection of PEDV in the field as well as in less well-equipped laboratories.
Molecular characterization of *Malassezia pachydermatis* isolates causing canine external otitis

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*Malassezia pachydermatis* has been commonly associated as a causal agent of canine external otitis in around 70.5% of the cases (1). Its diagnostics has traditionally been based on microbiological methods for phenotypical character determination; however, currently molecular biology tools have contributed to solve the disadvantages of conventional methods. The objective of this work focused on molecular species characterization of *Malassezia* isolates obtained from clinical samples of dogs with external otitis. Twenty isolates phenotypically identified as *M. pachydermatis, M. furfur* and *Malassezia* spp. were characterized by PCR amplification and sequencing of 5.8S (ITS3 - ITS4) (2) and 26S (D1 – D2) (3) rDNA regions, restriction with AluI, CfoI, and BstF5I enzymes, and phylogenetic analysis.

All isolates were identified as *M. pachydermatis* by both methods, obtaining a better phylogenetic resolution with 5.8S rDNA region. The 26S rDNA region restriction analysis with BstF5I enzyme showed 2 different restriction patterns, one with 3 bands of 311, 186, and 61 bp (60% of isolates) and other with 2 bands of 505 and 61 bp (40% of isolates). In conclusion, *rDNA sequencing and PCR-RFLP equally allowed identifying the canine Malassezia isolates at specie level. Also both techniques revealed the intraspecific genetic variability of M. pachydermatis isolates.*

**References**

Preliminary validation of the ID Screen® PEDV Indirect ELISA

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Porcine epidemic diarrhea (PED), caused by PED virus (PEDV), is an infectious and highly contagious viral disease of pigs characterized by severe diarrhea, vomiting, and dehydration. Detection methods to confirm PEDV infection include virus isolation, direct fluorescent antibody (FA), test or PCR. Antibodies may be detected by immunoperoxidase assay (IMPA), immunofluorescence assay (IFAT), viral neutralization test (VNT), or ELISA. ELISA offers the advantage of being cost-effective and easy to implement for high throughput testing. IDvet has developed an indirect ELISA based on a recombinant nucleocapsid, the ID Screen® PEDV Indirect ELISA.

Diagnostic specificity, evaluated on 512 sera from areas where the virus has not been reported in recent years, was 99.2% (IC95% 98.0; 99.7). Sensitivity was evaluated on 33 IFAT-positive sera; 30/33 samples were found positive by the IDvet ELISA. Testing of additional positive samples is underway. Global correlation with IFAT was 98%. The test detected animals experimentally-infected with the CV777 strain between 14 and 21 days post-infection.

Preliminary validation studies indicate that the ID Screen® ELISA is an efficient tool for disease surveillance and epidemiologic studies.
093 Effective detection and surveillance of antibiotic resistance genes from food samples and potential fertilizer sources using qPCR technology

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One potential source of acquiring antibiotic resistance genes is through the food supply. Both livestock and feed may acquire antibiotic-resistant bacteria through different mechanisms. For example, one source of fertilizer is the end-product from waste-water treatment plants that have treated water exposed to fecal microbiota, which is a potential reservoir for antibiotic-resistant genes. Livestock can then be exposed to antibiotic-resistant bacteria through feed where fertilizer from waste-water treatment plants has been used. This, in addition to increasing administration of antibiotics to livestock can lead to food as a potential source of antibiotic resistant genes. This may lead to horizontal gene transfer to pathogenic enteropathogens leading to drug resistance in humans, therefore highlighting the importance of surveillance and prevention of antibiotic resistance genes in food.

Real-time PCR methods have proven effective for the detection of antibiotic resistance genes, and PCR array technology allows the detection of a large number of genes in a single PCR run. Therefore, in this study, an antibiotic resistance gene identification PCR array was developed that allows for rapid screening of a range of antibiotic resistance genes present in a sample. The PCR array contains 5′ hydrolysis probe assays (primer and dual-labeled probe sets) that uniquely target 87 antibiotic resistance genes. All assays exhibited low-end sensitivity between 5–80 copies and a linear dynamic range of at least 5 orders of magnitude.

Because the gut is known to act as a reservoir for antibiotic resistance genes, a small-scale research study was performed on 5 stool samples isolated from healthy adult humans using the antibiotic resistance gene identification PCR array. All 5 samples had \textit{ermB} and \textit{mefA}, and 3 of the samples were positive for \textit{tetA}. Another study was done to determine the diversity of antibiotic resistance genes in municipal biosolids by analyzing the genes using Antibiotic Resistance Genes Microbial qPCR Array (catalog number: BAID-1901Z) using DNA extracted from belt-filter press cake sewage samples. There were 14 antibiotic resistance genes from different resistance. Further studies were performed in beef, chicken, vegetable and pork samples to determine presence of antibiotic resistance genes in food.

\textbf{In conclusion, PCR arrays can be effective tools for detection of antibiotic resistance genes from food samples and potential sources of fertilizer.}
A novel deltacoronavirus associated with wild birds detected during surveillance for *Porcine deltacoronavirus* and *Porcine epidemic diarrhea virus* in western Canada

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*Porcine epidemic diarrhea virus* (PEDV) is a coronavirus causing severe acute diarrhea, vomiting and dehydration in swine and is associated with high piglet mortality. In April 2013, the first case of PEDV was reported in the USA, and since then the virus has spread across much of North America resulting in significant economic losses to the swine industry. In August 2013, *Porcine deltacoronavirus* (PDCoV), which produced similar disease in pigs, was detected in the USA. In early 2014, PEDV and PDCoV were detected in Canada for the first time during outbreaks of piglet mortality. In Canada, detection of both PEDV and PDCoV is accomplished via real time PCR. The assays were developed in Guelph, Ontario, Canada.

PEDV can be readily spread by contaminated swine transport vehicles. It is assumed that PDCoV can be spread in a similar manner. Environmental surveillance of high traffic pig sites such as assembly yards and abattoirs has been used in early detection and control of PEDV in eastern Canada. Frequent environmental surveillance sampling occurs in western Canada. The western Canadian provinces of British Columbia, Alberta and Saskatchewan have remained free of both diseases up to this time.

In October 2014, environmental surveillance samples from a high traffic pig site in Alberta reacted to the PDCoV real-time PCR assay. No infected pigs or farms were found during the trace-back investigation. Despite extensive clean-up efforts and improved biosecurity at the site, environmental samples reacted positively to the assay again. Fecal and intestinal samples from pigeons and sparrows resident at the facility were sampled and tested to determine if birds could be a source of the virus or environmental contamination. These samples yielded positive results with Ct values between 30 and 37. The positive avian samples were sent to the National Centres for Animal Disease (NCAD) in Winnipeg, Manitoba for characterization. **Partial sequencing indicated a novel avian deltacoronavirus that was reacting to the PDCoV real-time PCR test.**

Detections of PDCoV in environmental samples by real-time PCR methods should be interpreted with consideration to possible contamination of samples with bird feces unless assays are specifically developed and validated to only detect PDCoV.

**Refinement of the real-time PCR test to distinguish between avian-associated and porcine deltacoronaviruses is under development.**
Evaluation of different commercially available ELISAs for serologic analysis of Aujeszky's disease in serum samples from wild boars

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Aujeszky's disease causes significant economic losses to the swine industry. Despite control and elimination of the disease in domestic pigs, wild pigs threaten to cause reintroduction of infection as they can act as a virus reservoir. The aim of this study was to evaluate 5 commercially available kits for detecting antibodies against Aujeszky's disease virus (ADV; Suid herpesvirus 1 [SuHV1]) in serum samples from wild boars (CIVTEST® SUIS ADV, CIVTEST® SUIS ADVgE, CIVTEST® SUIS ADVgB and 2 other commercially available competitive ELISAs for anti-gE; ELISA 1 and anti-gB; ELISA 2). The study was conducted using 74 boar serum samples classified into 4 groups according to their epidemiologic situation: (1) PCR+ / ELISA 1+; (2) ELISA 1+; (3) PCR- / ELISA 1-; and (4) vaccinated with a deleted vaccine.

All samples from animals in group (3) were negative in all ELISAs. Similarly, all animals vaccinated with a vaccine that has the deleted gE tested positive for gB-based ELISAs or in total antibodies (CIVTEST® SUIS ADV, CIVTEST® SUIS ADVgB and ELISA 2), and negative for those based on gE (CIVTEST® SUIS ADVgE and ELISA 1). The PCR+ / ELISA 1+ group yielded 64% positive in all ELISAs, whereas in the ELISA 1+ group this percentage fluctuated between 35% and 47%. The indirect ELISA, CIVTEST® SUIS ADV, yielded sensitivity and specificity results comparable to CIVTEST® SUIS ADVgB and ELISA 2 in all groups analyzed.

The high kappa values obtained in this study, both between the two ELISAs against gE as well as those for the 2 ELISAs against gB, indicate that the diagnostic features of these kits are practically identical; thus, they have been demonstrated to be equally suitable for use, in each case, in serologic studies on samples from wild boars.
Serosurveillance studies of rabies neutralizing antibodies in Nigerian fruit bat, *Eidolon helvum*

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The etiological agent of rabies is a member of the *Lyssavirus* genus (*Rhabdoviridae* family and order Mononegavirales). Members of this highly neurotropic group of viruses are characterized by a linear, single-stranded, negative-sense, non-segmented RNA genome. Rabies is endemic in Nigeria and, as in many parts of Asia and Africa, remains an important public health and veterinary health threat. In Nigeria, the natural infection of bat species with lyssaviruses has not been well studied and hence the scarcity of information.

The present study endeavors to provide new information on the exposure and prevalence of lyssavirus neutralizing antibodies in bat species in this part of Nigeria through surveillance studies. In order to bridge this knowledge gap, the current study investigated the extent of rabies virus (RABV) neutralizing antibodies in sera obtained from fruit bats, *Eidolon helvum*, from the central Plateau and North-East Bauchi State. A total of 200 bat sera were collected from 6 different locations via the propatagial vein and immediately stored at 4°C. Serum samples were subsequently heat-inactivated and stored frozen. The sera were tested using a commercial ELISA kit (BioPro rabies ELISA Ab kit, Prague, Czech Republic). The sampling and ethical procedures were done in accordance with the principles of the Ethical approval was obtained from the National Veterinary Research Institute, Institutional Animal Use and Care Committee (IAUCC).

The current study confirmed the presence of RABV neutralizing antibodies (VNAs) in 6 (3%) of the 200 bat sera, demonstrating a low prevalence of antibodies in the bat population studied here. Previously, the presence of VNAs in sera of fruit bats from Ibadan Nigeria bears testimony that these species could be maintenance hosts for lyssaviruses. This study reports the evidence of rabies antibodies circulating in Nigerian fruit bats and confirms results from other studies albeit a low prevalence of VNAs. In vampire bats species in Latin America, 72% of the serum samples were positive. In order to expand this study, the same panel of samples will be tested for neutralizing antibodies to members of phylogroups II and III, viruses that have been shown to lack cross-neutralization with RABV (member of phylogroup I). Only then will we be able to understand better the exposure of the lyssaviruses (rabies and rabies-related) in bat species within the country.

**Reference**

First case of pulmonary bovine tuberculosis in a free-living fallow deer in northwest Italy

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*Mycobacterium bovis* is the causative agent of bovine tuberculosis (bTB) and it is characterized by a wide host range, including deer. In Europe, *M. bovis* infection has been reported in red and fallow deer and less frequently in roe deer. Hence, deer may have an important role in bTB epidemiology. In Piedmont Region (northwest Italy), a regional monitoring plan to control the wildlife health status has been active since 1997 and enforced since the beginning of 2012. Passive surveillance has never detected bTB in wild ruminants found dead, euthanized because of injury or in poor condition, or in hunted game species.

This report describes the first case of *M. bovis* infection in a free-living fallow deer. The animal was euthanized because of its poor body condition. Postmortem examination revealed gross lesions in the thoracic cavity, characterized by a few encapsulated necrotizing nodules on dorsal and caudal lung and a small single mineralized focus in a peribronchial lymph node. Lesions in the lungs and lymph nodes were consistent with mycobacterial infection confused by the presence of a severe parasitic bronchopneumonia due to lungworms.

On histopathology, lungs had severe unencapsulated granulomatous and necrotizing multifocal to coalescing inflammation with rare Langhans giant cells. Lymph node showed multifocal areas of necrosis with mineralization surrounded by epithelioid macrophages, lymphocytes, plasma cells, neutrophils, and rare Langhans giant cells, enclosed partly or completely by a thin capsule. The lesions had numerous acid-fast bacilli. *M. tuberculosis* complex genome was directly detected from homogenized tissue samples using a hemi-nested PCR-based protocol targeted on the element insertion IS6110 and performed in-house.

Acid-fast organisms were isolated from lesions following 4 days of incubation using an automatic liquid system (Versatrek System, Thermofisher, Oxoid) and 12 days on solid media, then identified by means of multiplex PCR based on simultaneous detection of RNAr16S sequence, insertion element IS986, and mpt40 gene. The *M. bovis* strain isolated was further characterized by spoligotyping and VNTR typing (ETR A,B,C,D,E) as SB0120 45533. SB0120 spoligotype associated with 45533 VNTR profile is one of the most commonly isolated since 2003 in cattle herds of our region. In 2008, a homologous strain was also isolated from a water buffalo with extensive bTB lesions in a zoo, not far from the site of finding the infected fallow deer.

The poor condition of the animal was also attributable to heavy parasitic infestation and salmonellosis. Tubercular lesions were detected only in the lungs and associated lymph nodes, suggesting an airborne infection. Due to the presence of other about 20 free-living fallow deer, it is needed to improve the surveillance and avoid the spread of infection out of the group.
Characterization of PCV-2 in wild boars and their comparison with isolates of domestic pigs originating from Slovakia

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Porcine circovirus 2 (PCV-2) has been associated in domestic pigs with PMWS. Whereas PCV-2 is often detected in domestic pigs, knowledge of the PCV-2 infection rates in wild boars is still insufficient. To better understand the epidemiology of PCV-2 infections in the pig population, the distribution and genetic characterization of virus in wild boars was studied in this work. In addition, the comparison with data in domestic pigs was also performed. A total of 194 tissue homogenates (spleen, kidney, tonsils) of wild boars hunted during 2012 in 64 of 79 districts representing all 8 administrative regions of Slovakia were tested. The collection was composed of 118 samples originating from wild boars <1 year of age and 76 that came from animals >1 year of age.

Total DNA was extracted from 200 µL of homogenates using Chelex 100. PCV-2 DNA was detected by PCR using the CF8/CR8 primer pair flanking a 264 bp fragment from ORF2 (1). For phylogenetic analysis, the entire ORF2 region in length 702 was chosen. To obtain the complete ORF2 sequences, a nested-PCR for amplification of a 759 bp fragment was performed (2). Phylogenetic trees were constructed by the neighbor-joining method using MEGA6 software.

The overall PCV-2 prevalence in wild boars was 43.8%. The findings indicated moderate regional differences in its distribution since virus prevalence ranged from 31.3% to 56.3%. More young wild boars (48.3%) were infected with the virus compared to 36.8% of animals older than 1 year.

Among 20 PCV-2 nucleotide sequences of complete ORF2, 91.2% – 100% similarity was observed. They were 91.3% – 100% similar to domestic pig isolates originating from Slovakia. The phylogenetic tree constructed on the basis of alignment of 702 bp long ORF2 sequences revealed that most of the PCV-2 variants analyzed in Slovak wild boars (n = 17) belonged to the 1A/1B cluster of PCV-2b genotype. Three sequences were clustered into PCV2a, cluster 2D. Recently we showed (2) that similar clustering into PCV-2b-1A/1B and PCV-2a-2D was also observed with isolates originating from domestic pigs in Slovakia. However, virus prevalence in wild boars was significantly lower compared to domestic swine in Slovakia, where 64.2% prevalence was established. Taking into consideration that intensive pig farming, which allows no or very limited direct contact of both species, is predominant in Slovakia, the role of PCV-2 exchange between farmed and free-living animals in not clear.

Acknowledgement. This work was supported by VEGA grant No 1/0342/14.

References

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The purpose of this work was to evaluate the quality of oral rabies vaccination in the buffer zone on the border of Russia-Finland in the period 2009-2013. For oral immunization of wild animals, a vaccine based on attenuated strain RV-97 was used in 2010, a vaccine "Rabivak-O/333" (modified strain ERA G333) production of OAO "PZB" was used in 2011-2013. Vaccination of ~40,000 doses of oral rabies vaccine is applied twice every year in the territory of the Republic of Karelia.

We used pathology material (complex samples - brain, blood, branch of the mandible) from animals in the buffer zone of the Republic of Karelia in the period 2009 to 2013. The circulation of the rabies virus in wild populations of the target species (fox, raccoon dog) can be interrupted if the number of animals with a protective level of anti-rabies antibodies (seroprevalence) not less than 70%. Based on rabies virus neutralizing antibodies in the specified period, the level of seroprevalence was: 2009 - 80%, 2010 - 60%, 2011 - 65%, 2012 - 67.9%, 2013 61.1%. The percentage of animals with a protective level of antibodies was maintained for the last 5 years at a consistently high level. Information about the palatability of the oral vaccine can control the correctness of its distribution in the habitat of the target species of animals. The figure for the reporting period was as follows: 2009 - 7.7%, 2010 - 23.3%, 2011 - 33.3%, 2012 - 13.3%, in 2013 - 8.1%. The relatively low levels of vaccine palatability is not clear. However, international practice shows that a strong correlation exists between the levels of palatability and seroprevalence.

During the 5-year period 2009-2013, the number of animals investigated with a protective level of anti-rabies antibodies was maximal in 2009 (80%), in other years, this figure hovered close to 70% (60% - 67.9%). The achieved performance of oral rabies vaccination of wild animals and the lack of rabies cases testifies to the effectiveness of rabies vaccination in the Republic of Karelia.
100 Prevalence and molecular characterization of *Trichinella* sp. in wolverines from the Yukon

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Wolverines (*Gulo gulo*) are an economically important species in northern Canada because of their valuable fur. In northwestern North America, wolverines are undergoing increasing pressure from climate change, land use and developments, and overharvest. Their predatory and scavenging lifestyle predisposes wolverines for exposure to foodborne parasites such as *Trichinella* spp., a zoonotic nematode. The objective of the present study was to determine the prevalence and genotypes of *Trichinella* spp. in wolverines in the Yukon Territory.

Tongues of wolverines were artificially digested to detect and recover first stage larvae (L1). The species and/or genotype identity of L1 were determined by multiplex PCR. Fifty seven of 72 examined wolverines were positive for larvae (overall prevalence 79%), which is comparable to prevalence in wolverine in other studies in northern Canada. Prevalence was higher in males (84.4%) as compared to females (70%). The highest proportion of positive wolverines were collected in Yukon Southern Lakes ecoregions (14 wolverines) followed by Ruby Ranges (13) and MacKenzie Mountains ecoregions (8). Mean intensity was 21.87 larvae per gram of muscle tissue (LPG) (SE 3.5; range 0.2-134.6), which is high relative to other reports; the intensity of *Trichinella* spp. in wildlife generally ranges from 0.1 to 10 LPG. Out of 36 samples genotyped, T6 was detected in 29 (80.56%), *T. nativa* in only 3 (8.34%), and mixed *Trichinella* T6 and *T. nativa* infections were detected in 4 (11.12%) wolverines.

The high prevalence of *Trichinella* spp. infection among wolverines suggests that wolverines should be considered as an indicator species for *Trichinella* transmission in the Canadian Arctic.

*Graduate student presenter*
101 Value of microscopic blood smear evaluation in predicting disease severity and outcome in adult equine emergency admissions

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The complete blood count (CBC) is an essential part of the diagnostic workup, especially in emergent care. Many private veterinary practices have in-house hematology analyzers and routinely run in-house CBCs, however, microscopic evaluation of the blood smear is infrequently or inexpertly done (1). This can result in loss of clinically important information, in particular, the presence of band neutrophils and toxic change. We investigated the value of microscopic review of blood smears from 105 adult horses admitted for emergencies to a private equine practice near Calgary, Alberta, Canada. Inclusion criteria were a CBC and blood smear from the time of admission and age 1 year or older. Upon admission, a complete history was collected and a physical exam performed, including evaluation for systemic inflammatory response syndrome (SIRS) as a measure of disease severity. Cases were classified as gastrointestinal or non-gastrointestinal emergencies, and gastrointestinal cases were further divided into colic or colitis cases. Outcome was recorded for each case. Microscopic blood smear evaluation was performed by a board-certified clinical pathologist and focused on the detection of band neutrophils and toxic change. When band neutrophils were present, a 100-cell leukocyte differential count was performed. Band neutrophils were considered increased if they were >3% (i.e., a left shift was present). Toxic change, when present, was graded in a semi-quantitative manner on a scale of 0-9 (2). Toxic change was considered abnormal if the grade was >3.

Horses that died had both a higher percentage of band neutrophils and a higher grade of toxic change compared to horses that survived, as did SIRS cases compared to non-SIRS cases. Both increased percentage of band neutrophils and higher grade of toxic change on admission were associated with an increased risk of death for all emergency admissions and for colic cases. These associations demonstrate the value of clinically relevant information that may be missed without microscopic blood smear review. The clinician may choose a more aggressive course of treatment based on the presence of increased bands or toxic change at admission, which could potentially lead to a more positive outcome.

References
The purpose of this study was to describe the epidemiology of lead poisoning in Western Canadian cattle over the 16-year period of 1998 to 2013 and to determine background concentrations of lead in bovine tissues. Case records from Prairie Diagnostic Services in the Western College of Veterinary Medicine identified 517 cases of lead intoxication over the investigational period. Poisonings were influenced by year (p < 0.0001) and month (p < 0.0001). Submissions were greatest in 2009, 2001, and 2006 (n = 82, n = 60, and n = 52, respectively). The majority of poisoning events were observed during May, June, and July (82.7% of all cases; n = 322). Poisoning was most frequent in female cattle (69.6%; n = 197) and in animals <6 months of age (n = 189; p < 0.0001). Beef breeds were most often poisoned, of which Angus and Charolais breeds were predominant (n = 154, n = 169). Mean toxic levels of lead in the blood, liver, and kidney were 1.28 ± 1.71 mg/L, 34.3 ± 52.6 mg/kg wet weight, and 54.5 ± 40.3 mg/kg wet weight, respectively. Mean background concentrations of lead in the blood, liver, and kidney were 0.038 ± 0.003 mg/L, 0.16 ± 0.63 mg/kg wet weight, and 0.41 ± 0.62 mg/kg wet weight, respectively.

Occurrence of acute and subclinical lead poisoning in cattle is an ongoing problem in Western Canadian cattle operations. Frequency of poisoning events can be reduced through proper disposal of lead-containing wastes (i.e., lead-acid batteries, lead-based paints) and the continuous monitoring of herds potentially exposed to lead. Post-exposure assessment of animals that survived an episode of lead poisoning is critical. In addition, this assessment is equally, if not more, important in asymptomatic lead-exposed cattle. Normal background concentrations reported in this study are likely the largest documented database recorded in the literature. These values have substantial practical importance for veterinary practitioners and regulators.

References

*Graduate student presenter
103 Smartphone app development for animal health laboratory submissions: efficiencies for the veterinary practitioner and the diagnostic laboratory

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Mobile computing technology penetration in the veterinary profession has crossed the mass-use threshold; that is, most veterinary practitioners now take either a smartphone or a laptop/tablet computer with them on farm visits. Mobile applications are becoming increasingly available for data-intense tasks such as practice management, prescription writing, and herd health record keeping. Development of a mobile app for laboratory submissions would offer benefits and efficiencies for the submitting veterinarian as well as the veterinary diagnostic service provider.

Traditionally, the veterinarian fills out a paper form which accompanies the diagnostic submission. The information recorded on the form then must be manually entered into a laboratory information management system (LIMS) upon arrival. An electronic submission form would benefit both parties, as the electronic form can be completed more quickly and accurately than a paper form through the use of “smart” features such drop-down context-sensitive menus and cache of previous submissions. Eliminating the need for manual data entry on the laboratory side reduces labor costs and improves data quality and integrity through the elimination of transcription errors during manual data entry. Besides eliminating human errors, a mobile app can enforce completion of mandatory fields to ensure the collection of a complete dataset. There is a clear need to develop electronic submission form capacity for veterinary diagnostic service providers, particularly if the laboratory data is to be used for automated animal health syndromic surveillance on a regional or national level.

Based on feedback from veterinary practitioners, a successful laboratory submission app will need to have a simple user interface, fully utilize available on-board smartphone resources (e.g. built-in sensors, GPS and Wi-Fi radios, camera, voice recorder), and have a streamlined workflow to enable the user to complete a submission in under 2 minutes.
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