12th ANNUAL MEETING OF THE CANADIAN ANIMAL HEALTH LABORATORIANS NETWORK (CAHLN)

May 26-29, 2013

New technologies
Porcine infectious diseases

12e RÉUNION ANNUELLE DU RÉSEAU CANADIEN DES TRAVAILLEURS DES LABORATOIRES DE SANTÉ ANIMALE (RCTLSA)

26 au 29 mai 2013

Nouvelles technologies
Maladies infectieuses porcines

Hosted by / Reçu par
Faculté de médecine vétérinaire de l'Université de Montréal
Saint-Hyacinthe, Québec

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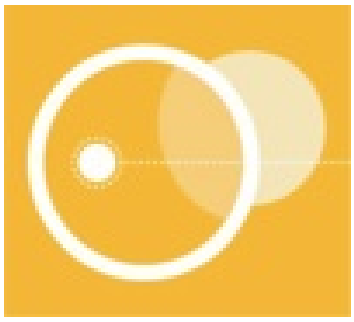

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(CAHLN)

May 26-29, 2013
Faculté de médecine vétérinaire de l'Université de Montréal,
Saint-Hyacinthe, Québec

INTRODUCTION

The CAHLN was established in 2002 to facilitate exchange of information on animal health diagnostic trends, techniques and research, to provide a venue for «networking» to identify common issues of concern, and to improve linkages among organizations and scientific staff involved in animal health diagnostic work in Canada.

The CAHLN is comprised of individuals across the wide spectrum of laboratory disciplines, including bacteriology, immunology, molecular biology, parasitology, pathology (anatomic and clinical), surveillance/ epidemiology, toxicology, and virology.

Previous annual meetings have been held in:

2002 – Ottawa (CFIA OLF)
2003 – Ottawa (CFIA OLF)
2004 – Guelph (Animal Health Laboratory, Ontario Veterinary College)
2005 – St-Hyacinthe (Faculté de médecine vétérinaire)
2006 – Ottawa (CFIA OLF)
2007 – Saskatoon (Western College of Veterinary Medicine / Prairie Diagnostic Services)
2008 – Ottawa (CFIA OLF)
2009 – Charlottetown (AVC – UPEI)
2010 – Calgary (UCVM)
2011 – Guelph (Animal Health Laboratory, Ontario Veterinary College, University of Guelph)
2012 – Winnipeg (CFIA – NCFAD – MAFRI)

The 2013 local organizing committee is comprised of: Estela Maria Cornaglia (Chair), Véronique Boyer, Jean-François Brodeur, Guy Fontaine, Véronique Allard, Donald Tremblay, Justine Desrosiers and Cécile Crost.

The 2013 Scientific committee is comprised of: Marie Archambault D.M.V., Marie-Odile Benoît-Biancamano D.M.V., Ph.D., Yasmine-Djaouida Chalal Ph.D. (ACIA), Julie-Hélène Fairbrother D.M.V., Josée Harel Ph.D. (GREMIP) and Carl Gagnon D.M.V., Ph.D.

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INTRODUCTION

Le RCTLSA a été créé en 2002 dans le but de favoriser l'échange d'informations sur les tendances, les techniques et la recherche en matière de diagnostic en santé animale; de fournir une occasion de «réseautage» afin de dégager des sujets de préoccupations communs dans ce domaine; et de faciliter les relations entre les organisations et le personnel scientifique dont le travail touche le diagnostic en santé animale au Canada.

Le RCTLSA comprend des personnes provenant de toutes les spécialités de diagnostic en laboratoire, incluant des spécialistes en bactériologie, en pathologie (anatomique et clinique), en immunologie, en virologie, en parasitologie, en surveillance /épidémiologie, en toxicologie et en biologie moléculaire.

Les réunions annuelles précédentes ont eu lieu à :

2002 – Ottawa (CFIA OLF)
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Le comité organisateur de la réunion de 2013 est composé de: Estela Maria Cornaglia (Chair), Véronique Boyer, Jean-François Brodeur, Guy Fontaine, Véronique Allard, Donald Tremblay, Justine Desrosiers and Cécile Crost.

Le comité scientifique de la réunion de 2013 est composé de: Marie Archambault D.M.V., Marie-Odile Benoît-Biancamano D.M.V., Ph.D., Yasmine-Djaouida Chalal Ph.D. (ACIA), Julie-Hélène Fairbrother D.M.V., Josée Harel Ph.D. (GREMIP) and Carl Gagnon D.M.V., Ph.D.

CAHLN Laboratorian of the Year
Prix du diagnosticien du RCTLSA

The Canadian Animal Health Laboratorians Network (CAHLN) awards a plaque annually to a laboratorian based on his or her noteworthy contributions to veterinary laboratory medicine in Canada. A nominee might be an outstanding diagnostician, educator, researcher, mentor of future laboratorians or other contributor to the field.

Le Réseau canadien des travailleurs des laboratoires de santé animale (RCTLSA) remet à chaque année une plaque à un diagnosticien pour ses contributions remarquables à la médecine vétérinaire de laboratoire du Canada. Un candidat peut être un diagnosticien exceptionnel, éducateur, chercheur, mentor de futurs diagnosticiens de laboratoire ou autre collaborateur du domaine.

Past Recipients / Récipiendaires des années passées :

2003 – Lloyd Spencer, CFIA
2004 – Ian Barker, OVC
2005 – Marcelo Gottschalk, FMV
2006 – John Robinson, MAL
2007 – John Fairbrother, FMV
2008 – W. D. G (Bill) Yates, CFIA
2009 – Gerald R. Johnson, AVC
2010 – Ted Clark, Calgary
2011 – Josepha Patricia DeLay, OVC
2012 – Mark Swendrowski, MAFRI

CAHLN Graduate Student Presentation Award

Prix de présentation d'un étudiant diplômé du RCTLSA

A plaque is awarded annually to a graduate student based on the quality of his or her presentation at the CAHLN annual meeting. Presentations are judged on the originality of the subject, contribution of the presentation to our knowledge base, the student's understanding, delivery of the topic, and his or her ability to deal with questions.

The award is presented at the conclusion of the CAHLN annual meeting.

Chaque année, une plaque est remise à l'étudiant qui a présenté la meilleure présentation dans le cadre de l'assemblée annuelle du RCTLSA. Les présentations sont jugées selon l'originalité du sujet, la contribution de la présentation à nos connaissances, la compréhension du sujet par l'étudiant et la qualité de sa présentation ainsi que sa capacité à répondre aux questions.

Le prix est décerné à la fin de la réunion annuelle du RCTLSA.

Past Recipients / Récipiendaires des années passées :

2003 – Sherry Andrews, WCVM
2004 – Noel Arrington, OVC
2005 – Guillaume Bruant, FMV
2006 – Yuanmu Fang, WCVM
2007 – Kathi Ellis, WCVM
2008 – Angela Catford, OVC
2009 – Raphael Vanderstichel, AVC
2010 – Guilherme Gomes Verocai, UCVM
2011 – Olivier Cote, OVC
2012 – Jason Struthers, WCVM

New for 2013! / Nouveau en 2013!

CAHLN Graduate Student Travel Awards

Bourse de voyage pour étudiants gradués du RCTLSA

The travel awards are intended to help defray the cost of travel and lodging for students traveling to the Annual CAHLN meeting from sites distant to the host site, encouraging student excellence in their laboratory discipline, developing presentation skills (verbal, written, organizational), and promoting networking of students with veterinary laboratorians. This year, funds are available for 2 travel awards of $500 each.

Trainees in a Master's, PhD or residency program enrolled in any discipline in veterinary laboratory medicine were encouraged to apply by submitting an abstract, a motivation letter, a resume and a support letter from their mentor.

Les bourses de voyage sont destinées à aider à couvrir les frais de voyage et d'hébergement pour les étudiants voyageant à la réunion annuelle du RCTLSA des sites éloignés du site d'accueil, en encourageant l'excellence des étudiants dans leur discipline de laboratoire, le développement des compétences de présentation (orale, écrite, organisationnelle), et favoriser le réseautage des étudiants avec les diagnosticiens des laboratoires vétérinaire. Cette année, les fonds sont disponibles pour 2 bourses de voyage de 500 $ chacune.

Les étudiants à la maîtrise, au doctorat ou dans un programme de résidence, inscrits dans n'importe quelle discipline en médecine de laboratoire vétérinaire ont été encouragés à appliquer sur ces bourses en remettant un résumé de la présentation, une lettre explicative, un curriculum vitae ainsi qu'une lettre d'appui de leur mentor.

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CAHLN/RCTLSA 2013
Program / Programme

Sunday, May 26, 2013 / Dimanche 26 mai 2013

08:15-17:15	**CAVP Annual Pathology Conference**
18:30-21:00	CAHLN/CAPV Evening Reception – *Complexe de diagnostic et d'épidémiosurveillance vétérinaires du Québec*. 3220, rue Sicotte, Saint-Hyacinthe, J2S 2M2
18:30-20:00	**CAHLN Meeting Registration**, reception and tour of the new *Complexe de diagnostic et d'épidémiosurveillance vétérinaires du Québec*
20:00-21:00	**Guest Speaker:** Grant Maxie – Still working on work-life balance!

Monday, May 27, 2013 / Lundi 27 mai 2013

07:45 – 15:00	**Registration Desk – Faculty of Veterinary Medecine**. Room 1134. 3200, rue Sicotte, Saint-Hyacinthe, J2S 2M2
08:30 - 08:40	**Official Opening and Welcome** **Michel Carrier**, Dean of the Faculty of Veterinary Medicine, Université de Montréal
Session Theme:	**Diagnosis and Laboratory Research in Quebec** **Moderator: Yasmine Djaouida Chalal**
08:40 – 09:10	Diagnosis laboratories in Quebec. **Estela Cornaglia**. Faculty of Veterinary Medicine, Université de Montréal
09:10 – 09:40	Diagnostic en santé animale au labo de l'agence St-Hyacinthe. **Bianca Morel**. Canadian Food Inspection Agency.
09:40 – 10:10	La surveillance de la santé animale au Québec (Québec's Animal Health Surveillance). **Michel Major**. Ministère de l'Agriculture, des Pêcheries et de l'Alimentation du Québec (MAPAQ)
10:10 – 10:40	Pause / Exhibits
10:40 – 11:25	CRIPA: Research and diagnostic. **Josée Harel**. GREMIP- CRIPA. Faculty of Veterinary Medicine, Université de Montréal

11:25 – 11:45 Quality assurance by and for veterinary pathologists. **Grant Maxie**. AHL. Guelph.

11:45 – 12:00 CAHLN - Laboratorian of the Year

12:00 – 13:30 Lunch / Exhibits

12:30 - 13:30 CAHLN Business meeting. Room 1134

Session theme: New technologies

Moderator: Julie-Helene Fairbrother

13:30 – 14:00 New methods for sequencing. **Ken Dewar**, McGill – Genome Quebec.

14:00 – 14:30 Application of the MALDI-TOF MS in routine bacteriology. **Durda Slavic**. AHL. Guelph.

14:30 – 15:00 Nanotechnology applications in diagnostic laboratories. **Alexis Vallée-Belisle**. Directeur du Laboratoire de Biocapteurs & Nanomachines. Université de Montréal.

15:00 – 15:10 Sponsor's presentation. Biovet

15:10 – 15:40 Pause / Exhibits

15:40 – 16:00 Forensic animal disease investigations: An update. **David Silversides**. Université de Montréal.

16:00 – 16:10 Science on the spot

16:10 – 16:20 Sponsor's presentation. Qiagen

16:20 Adjourn

16:30 – 19:30 **CAHSN Executive Board meeting** (by invitation)

Tuesday, May 28, 2013 / Mardi 28 mai 2013

Session theme: Epidemiology

Moderator: Denise Bélanger

08:30 – 08:50 Validity of conclusions in population-based surveillance. **Farouk El Allaki**. Canadian Food Inspection Agency.

08:50 – 09:10 The role of diagnosticians in terrestrial animal disease surveillance. **Julie Paré**. Agence Canadienne d'inspection des aliments.

09:10 – 09:30 Estimating diagnostic sensitivity and specificity using the published Literature - a terrestrial animal disease example- bovine brucellosis. **Julie Paré**. Agence Canadienne d'inspection des aliments.

09:30 – 09:50 Surveillance, scenario tree models and claim of disease freedom at the national level. **Jette Christensen**. Canadian Food Inspection Agency.

09:50 – 10:00 Sponsor's presentation. Life Technologies

10:00 – 10:30 Pause / Exhibits

10:30 – 10:50 Approaches to ensuring data integrity and quality in surveillance using swine pseudorabies as an example. Jette Christensen. Canadian Food Inspection Agency.

10:50 – 11:10 Estimating disease freedom using scenario tree models-Trichinella. Jette Christensen. Canadian Food Inspection Agency.

11:10 – 11:30 Estimating disease freedom using scenario tree models- Canadian Notifiable Avian Influenza Surveillance System and NAI. Jette Christensen. Canadian Food Inspection Agency.

11:30 – 11:50 A benefit/cost financial management spreadsheet for veterinary laboratory emergency preparedness. **Maria Spinato**. AHL. Guelph.

11:50 – 12:00 Sponsor's presentation. Tetracore.

12:00 – 13:30 Lunch / Break

12:30 – 13:30 CDEVQ's visits

13:30 – 13:50 Determining the gold standard ante-mortem sample type for detection of *Brachyspira* in pigs. **Matheus Costa**. University of Saskatchewan.

13:50 – 14:10 Identification, molecular characterization and electron microscopy of a new *Hemoplasma* in Canadian swine herd and development of a real-time PCR assay for the diagnosis of the infections. **Donald Tremblay**. Université de Montréal.

14:10 – 14:30 Development of an indirect enzyme-linked immunosorbent assay for detection of serum antibodies to leporid herpesvirus-4. **Janet Sunohara-Neilson**. University of Guelph.

14:30 – 14:40 Sponsor's presentation. ESBE.

14:40 – 15:10	Pause / Exhibits
15:10 – 15:30	Detection of *Actinobacillus pleuropneumoniae* (App) serotype 1 in pigs by real-time quantitative PCR. **Sonia Lacouture**. Université de Montréal.
15:30 – 15:50	Caractérisation moléculaire des *E. coli* d'origine utérine chez la vache laitière postpartum. **Flavien Ndongo Kassé** - Université de Montréal.
15:50 – 16:10	Development of a new microarray-based diagnostic tool to detect novel viral pathogens involved in potentially devastating animal diseases. **Christian Bellehumeur**. Université de Montréal.
16:10	Adjourn
17:15	**Informal social get-together**
16:30 – 18:00	**National TSE Laboratory Network Meeting** (by invitation)

Wednesday, May 29, 2013 / Mercredi 29 mai 2013

Session theme: Porcine infectious diseases

Moderator: Dr Sylvie D'Allaire.

08:00 – 08:30	Sequencing complete virus of PRRS. **Carl Gagnon**. Université de Montréal.
08:30 – 09:15	PRRS diagnostic. **Jane Christopher Hennings**. University of South Dakota.
09:15 – 10:00	Update *Brachyspira* **John Harding**. University of Saskatoon.
10:00 – 10:15	Pause
10:15 – 11:00	Update *Mycoplasma hyorhinis*. **Maria Calvinio**, University of Minnesota
11:00 – 11:45	Update *Streptococcus suis* and APP. **Marcelo Gottschalk**. Université de Montréal
11:45 – 11:50	CRIPA. Josée Harel. Université de Montréal
11:50 – 11:55	SD au CDEVQ. Estela Cornaglia. Université de Montréal.
11:55 – 12:10	**Presentation of award:** CAHLN – Graduate Student Presentation
12:10	Adjourn

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Oral Presentations / Présentations orales

Quality assurance by and for veterinary pathologists

Grant Maxie

Animal Health Laboratory, Laboratory Services Division, University of Guelph, Guelph, Ontario, Canada N1H 6R8

The Animal Health Laboratory operates under a comprehensive quality management system, and is accredited by various bodies, including the American Association of Veterinary Laboratory Diagnosticians (AAVLD), and has an expanding list of tests on our ISO/IEC 17025:2005 scopes of accreditation with the Standards Council of Canada and the Canadian Association for Laboratory Accreditation. Within the AAVLD standard, which is patterned on ISO 17025, element 5.2.1 states that "The laboratory shall ensure the initial and ongoing competence of all laboratory personnel to do their assigned work." This task is accomplished more easily with proficiency testing of staff that generate numerical data, rather than with personnel who generate subjective data, including anatomic and clinical pathologists.

Within the AHL quality program, all staff, including pathologists, follow processes documented in standard operating procedures (SOPs) housed within our electronic document control system (Omni-Assistant). The specific AHL quality assurance (QA) plan for pathologists includes third-party proficiency testing [Veterinary Laboratory Association (VLA) for histopathology, hematology, and cytopathology; Animal Health and Veterinary Laboratories Agency (VETQAS) for cellular antigen immunohistochemistry (IHC); and the National Veterinary Services Laboratory (NVSL) for infectious antigen IHC; the director's quarterly review of completed cases by individual pathologists; bi-weekly peer review of randomly selected cases; and twice-weekly gross pathology and histopathology rounds. The bi-weekly peer review of cases involves circulation of glass slides and review of all documentation on the case by each pathologist, followed by group review of each case. Pathologists in our regional laboratory in Kemptville, Ontario, are linked to Guelph pathologists via teleconference, and fully participate in QA activities. The above QA activities are documented, are auditable, and contribute to continuous improvement within our quality program.

Durda Slavic, DVM, MSc, PhD

Dr. Slavic obtained her DVM degree from Faculty of Veterinary Medicine, University of Zagreb, Croatia in 1994. After graduating she worked for 2 years in veterinary inspection before coming to Canada. She moved to Canada in 1996 and in 1997 enrolled into a graduate program at the Department of Pathobiology at the University of Guelph where she earned her MSc and subsequently her PhD degree in Veterinary Microbiology. Currently Dr. Slavic is working full time for the Animal Health Laboratory supervising Bacteriology laboratory. She is in charge of clinical microbiology, mastitis, hatchery and Johne's disease testing.

Application of Matrix-Assisted Laser Desorption Ionization Time-of-Flight Mass Spectrometry (MALDI-TOF MS) in routine clinical bacteriology laboratory

Durda Slavic, Animal Health Laboratory, University of Guelph

Currently routine clinical diagnostic bacteriology still heavily relies on the classical biochemical methods for bacterial identification. These methods usually require 12 or more hours for their completion. In contrast Matrix-Assisted Laser Desorption Ionization Time-of-Flight Mass Spectrometry (MALDI-TOF MS) bacterial identification can be achieved within minutes. A single bacterial colony from overnight culture, grown on different media types, is simply smeared on a MALDI-TOF MS stainless steel 96 well target and overlaid with matrix. The matrix/sample mixture is loaded into the MALDI-TOF MS instrument and protein spectra are generated. These protein spectra are then compared to the spectra in the database which are unique for each bacterial species and the bacterial identification is given. Initially MALDI-TOF MS has been used in research but more recently it gained popularity among diagnostic labs across North America. As with every new technology several factors must be considered before introducing it to routine diagnostic testing including cost/benefit analysis, validation of the system etc. These factors will be discussed in addition to the use of MALDI-TOF MS as a part of routine AHL clinical microbiology diagnostic testing.

Alexis Vallée-Bélisle, PhD

Dr Alexis Vallée-Bélisle est professeur adjoint au Département de chimie de l'Université de Montréal et Directeur du Laboratoire de Biocapteur & Nanomachines depuis novembre 2012. Il est le récipiendaire du Canada Rising Star in Global Health (2012) et de l'"International Center Material Research Award –NSF" (2011). Après un baccalauréat en biochimie (1997) et une maîtrise en chimie (2000) à l'Université de Montréal, Dr Vallée-Bélisle complète son Ph.D. en biochimie sous la supervision du Pr Stephen Michnick à l'Université de Montréal sur le développement de nouvelles technologies permettant de caractériser les mécanismes d'auto-assemblage des nanomachines naturelles (protéines). De 2008-2012, il poursuit ses études postdoctorales au Center for Bioengineering de l'University of California, Santa Barbara sur le développement de biocapteurs électroniques. Ses recherches actuelles portent sur le développement de biocapteurs électroniques pour la détection rapide (moins de 5 minutes) de différents marqueurs moléculaires et sur le développement de nanomachines pour la délivrance ciblée de médicament.

Bio-Inspired Sensors for the Rapid, Easy-to-Use Detection of Antibodies and other Disease Markers

All creatures, from bacteria to humans, monitor their environments in order to survive. They do so with biomolecular switches, made from RNA or proteins. Inspired by this powerful natural technology, our laboratory has recently developed several new artificial biosensors that use fluorescent or electrochemical nanoswitches made from nucleic acids, peptides or proteins to detect chemicals ranging from small molecules to various macromolecules such as antibodies. These sensors are rapid (less than 5 minutes), reagentless, and selective enough to be employed directly in whole blood. In my talk, I will explain how we design and build these nanoswitches and will detail our efforts to adapt them into the next generation of diagnostic testing.

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Forensic Animal Investigations: An Update

David W. Silversides, Isabelle Daneau - Veterinary Genetics Laboratory, Diagnositic Services

Forensic science is the application of scientific knowledge to legal questions and problems. Although forensic medicine is a well established discipline for legal questions concerning human medical situations, it is much less developed within the context of veterinary medicine. In addition, expertise in animal forensic science has traditionally been from a pathology perspective. Advances in DNA technologies and availabilities of animal DNA and genomic sequences are now changing this situation, and are presenting new opportunities for veterinary diagnostic labs. At the Faculty of Veterinary Medicine in St-Hyacinthe, Québec, we have established the Laboratory of Veterinary Genetics, where we address questions of animal genetic disease, identification, and forensic cases. For the diagnostic lab, forensic cases involving animals must address sample procurement and preparation, sample identification as well as chain of possession. The preservation and biological integrity of the sample are important to the questions that need to be addressed. Sample contamination, real and potential, are concerns for the diagnostic laboratory. Forensic cases for the Laboratory of Veterinary Genetics usually come from government agencies, including the Québec provincial police (Sureté de Québec), the Canadian Border Security Agency, the Ministères de l'Environnement, de Resources naturelles, de l'Agriculture (MAPAQ) de Québec, and MAFRI (Manitoba) in conjunction with the RCMP. The diagnostic lab must conform to the established procedures, protocols and requirements of each of these outside interests. DNA techniques can be quite useful in resolving forensic cases involving animals, although cases tend to require individual rather than standardized approaches. Specific questions to be resolved include the presence (or absence) of animal material, the species identification of animal material, the genetic identification of individual animals, the parentage of individual animals, and the confirmation (or elimination) of suspect individual animals. Techniques include analysis of mitochondrial DNA, and microsatellite analysis of genomic DNA.

Validity of conclusions in population-based surveillance

Farouk El Allaki and Julie Paré - Canadian Food Inspection Agency, Epidemiology and Surveillance Section, Saint-Hyacinthe, Québec, Canada

The word 'Surveillance' was originally derived from the French verb 'Surveiller' which literally means 'to watch over'. ''Population-based Surveillance'' is a continuous and dynamic process of close observation of a specific population that includes: ongoing data collection, analysis, interpretation, knowledge production, and timely information dissemination to an appropriate audience. It is a repetitive process aimed at producing targeted information.

Population-based surveillance has several goals– demonstrating that a disease is not present; detecting presence of a disease; establishing the level of disease occurrence, or assessing effectiveness of a disease control strategy. The design of the surveillance has to be coherent with the objective of surveillance, defined before surveillance implementation, and composed of the following elements: target population and population hierarchy definition, case definition, data collection plan, sampling plan, data analysis plan, communication plan, validation plan, resource plan, and audit plan.

Inferences made from surveillance results should be clearly identified during the design phase. Validity of the surveillance results and conclusions relies on the validity of the surveillance design and implementation. End-users of a surveillance system will be more likely to consider the results of surveillance when they are confident that what it is reporting has internal validity and, when necessary, external validity.

Internal validity indicates whether or not, the surveillance provided the ''correct'' estimates or answer for the source population (e.g. list of mature cattle slaughtered in federally inspected plants). Source population represents the immediate population for which the surveillance conclusions are to be used and from which a sample (e.g. cattle tested) is to be selected. In order to make a valid statistical inference, the sample should be representative of the source population. Internal validity relates to the surveillance design and assesses the presence of systematic errors.

External validity relates to the ability to make correct inferences to populations beyond the source population. The Target population (e.g. mature cattle in Canada) refers to a population to which surveillance conclusions are to be extrapolated. Ideally, the source population is the same as the target population, but often this not the case. The difference between the source and target population has to be described and documented.

A well-designed surveillance system may be able to make valid conclusions about the source population but these conclusions may not be extended to the target population if the two populations are different. Inferences from surveillance results must be justified and documented to ensure conclusion validity.

The role of diagnosticians in terrestrial animal disease surveillance

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Surveillance is defined by the OIE as the ongoing, systematic collection, collation, analysis and interpretation of animal health data, and dissemination of results for decision making. Surveillance in CFIA has focussed on foreign animal disease and has taken on many forms in CFIA, from periodical surveys to targeted surveillance. The purpose of surveillance has been to preserve animal and human health and to maintain market access.

The last few years have brought new processes and methodologies for surveillance. Collaborative surveillance has emerged, which involves federal and provincial veterinary authorities, as well as industry. A formal framework of ongoing planning & design, implementation, analysis & interpretation and reporting is used.

Diagnosticians play a key role throughout the surveillance process. In the planning stages, diagnostic support is essential to set a solid case definition, to ensure a realistic diagnostic testing scheme is used, and to provide test performances. In the implementation phase, the laboratory is involved in testing obviously, but also in troubleshooting and day-to-day quality assurance. In the analysis and interpretation phases, diagnosticians are involved in interpretation of results, and follow up testing when needed. Diagnostic specialists are also involved in the critical reviewing and authorship, for peer reviewed or internal reporting. For all stages of surveillance, a consultative and collaborative approach is necessary to ensure scientific validity of the diagnostic process and ultimately of the surveillance system.

Alternative analytical techniques have allowed the incorporation of many components of surveillance into a surveillance system, which allows estimation of the probability of freedom from a given disease. Use of scenario tree modelling builds on the sensitivity of detection of a disease, which is intricately linked to tests used and their sensitivity.

Surveillance must be flexible, ready to adjust for new diseases, environmental changes such as in the epidemiology a disease, in test availability or performance, emerging issues or disease prioritisation. The collaborative framework is appropriate for addressing these issues.

Estimating diagnostic sensitivity and specificity using the published literature- a terrestrial animal disease example- bovine brucellosis

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Canada has successfully eradicated Brucella abortus from its domestic herd and is free of the disease since 1985. An ongoing surveillance program has contributed to maintaining our status of freedom from this reportable disease.

We are currently using scenario tree modeling to determine the probability of freedom from brucellosis and have incorporated multiple tests for which we needed to estimate sensitivity. Our objective was to critically review the literature to extract data pertinent to the canadian population. We will present the results for the buffered plate agglutination test (BPAT), a test used worldwide for the detection of antibodies for brucella spp.

We reviewed 14 publications presenting performance of the BPAT and we recorded reported sensitivity, the number of true positive cattle on which sensitivity was computed and the criteria to determine their positive status. We also recorded reported specificity, the number of true negative cattle on which specificity was computed and the criteria to determine their negative status. Analytical approach (Bayesian or traditional) and test protocol details (cutoff, deviation from standard protocol used in CFIA laboratories) were recorded. Vaccination has been prohibited in Canada since 2000. For the analysis, all vaccinated cattle were excluded and if necessary sensitivity and specificity were recomputed using only non-vaccinated animals.

Two articles were rejected because: 1) Data was the same as reported in another article and 2) Article was not clear on vaccinal status of animals tested. Data from the 12 articles were compiled and a beta distribution was calculated for each study. The 2.5th, 25th, 50th, 75th and 97.5th percentiles were calculated and summary estimates were weighted on the number of animals used to calculate sensitivity in each article. Graphical representation of the estimates, as well as the overall estimated low, most likely and high estimate of sensitivity will be presented.

Overall, this method appeared to be an appropriate method to pool multiple sensitivity estimates into one estimate. Moreover, this method provides data necessary to build a probability distribution that will account for uncertainty around the sensitivity point estimate, for use in scenario tree modeling.

Surveillance, scenario tree models and claim of disease freedom at the national level

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The objective is to explain how surveillance and scenario tree models to estimate probability of freedom fit into a country's claim of freedom from animal disease.

When disease is present in the population the motivation for conducting surveillance can be decision support for disease control activities. The goal can be baseline study or monitoring changes in prevalence. When disease is absent from a population, the motivation for surveillance can be market access or early warning, It is important to recognize that the surveillance needed for the different motivations and goals are very different. Here I will focus on market access and the goal to prove freedom from an animal disease. I refer to this type of surveillance as "surveillance to confirm freedom".

The scenario tree model approach is the modern epidemiological analysis for surveillance to confirm freedom [1]. The strength of the method is that it can combine data from very different surveillance activities and it estimates the probability of the population being free at a given low prevalence. The scenario tree model is intuitively well explained by the scale diagram. This is the most intuitive representation of scenario tree models but a detailed clear and precise scientific presentation is needed to validate a model and to use the results in trade negotiations. Recently, it was suggested to present scenario tree models in a standardized way with (1) definitions to describe the objective of the model, (2) initial time period, (3) input parameters, (4) data, (5) model settings (6) outputs, and (7) validation [2].

Now a probability of freedom cannot stand alone when a country claims freedom. We also need to provide a thorough description of the context of the scenario tree model including: Epidemiology, nature and history of disease; description of the population, production methods; veterinary infrastructure and laboratory capacity; surveillance results including probability of freedom, coverage of population and geography; regular reporting; and audit results.

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Approaches to ensuring data integrity and quality in surveillance using swine pseudorabies as an example

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Modern epidemiological surveillance methods to confirm freedom from disease and support a country's claim of freedom can utilize data from different sources. Here, we give an example of how diagnostic test data from the CFIA's laboratory sample tracking system (LSTS) can be extracted and what it takes to prepare the data for scenario tree modeling and we used pseudorabies testing results as an example.

The nature of pseudorabies is that it will spread quickly in a herd of swine – we say it clusters in herds. Therefore it is critical to be able to count herds (or farms) and animals if we want to include diagnostic testing results from LSTS in our scenario tree models.

To extract the pseudorabies data from LSTS and create a database useful for the modeling was not trivial. First, we had to describe and decide on the structure of the data. For example, an operator (or swine producer) could have more than one premises (farms) where (s)he raised pigs that were tested for pseudorabies. We decided that it was feasible with the data from LSTS to have identifiers for "operator", "premises" and "sample" so that is what we hoped to count.

However, before we can start counting we need to check the data for (1) consistency, was "Mr Jones", "mr Jones" and "Jones" the same operator; (2) completeness, do we have the full address with postal code for all records; (3) obvious typos, there is no postal code "C1A" in Manitoba; and (4) missing data, if the sampling date was missing it was set to the submission date. To ensure data integrity and quality this data check must be fully documented.

We succeeded to create a pseudorabies database with 131 937 records of testing for pseudorabies based on LSTS data from 2007 to 2011. We were also able to count operators, premises and animals by province. The data will be included in the development of a scenario tree model to confirm freedom from pseudorabies in Canadian swine.

We conclude that LSTS data can be a valuable source of data for surveillance to confirm freedom from animal diseases but it takes a considerable effort to ensure adequate data integrity and quality.

Estimating Disease Freedom using Scenario Tree Models - Trichinella

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The objective is to use the Trichinella scenario tree model to illustrate how we proceed from the theoretical methodologies of a scenario tree model (described graphically) to a standardized description and reporting of a model [1].

In short the schematic diagram features a grocer's scale which has on one side water dripping into a bucket, to represent the continual risk of new introductions into the population (probability of introduction of Trichinella), and the faucet represents biosecurity (such as rodent control), regulating the flow of water. On the other side of the scale we have weights that represent the information gathered from past and present surveillance data (meat testing and surveys). The scale's needle shows the probability of freedom in the population after accounting for the balance between the risk of introduction and the evidence from surveillance activities.

We describe the Trichinella model using the standardized format. (1) definitions to describe the objective of the model: the case definition (CD) was detection of at least one Trichinella larva in a pig's meat sample, the time period (TP) was 1 year, the population was all market hogs shipped to federally inspected slaughter plants in Canada, the design prevalence (DP) was 0.01%; (2) initial time period: the first TP was 1997 and the prior probability of infection was 50%; (3) the input parameters were digestion assay sensitivity Pert(40%,75%,99.9%) and probability of introduction Pert(0.1%,3%,7%); (4) data about 28 000 hogs were tested annually from 1999 to present; (4) model settings: the model was developed in R programming language and 50 000 iterations were run (5) outputs: the posterior probability of freedom at the DP was presented graphically; and (6) the validation included: assessment of biological validity by model review by peers in industry (Canadian Swine Health Board) and government (Canadian Food Inspection Agency), technical validity by assessing estimates from running the model in Excel (with Poptools add-on), R programming language, and Scenario trees to quantify freedom from disease (http://freedom.ausvet.com.au), and peer-reviewed by publication in Preventive Veterinary Medicine.

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Estimating disease freedom using Scenario Tree Models - Canadian Notifiable Avian Influenza Surveillance System and NAI

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The objective is to present the CanNAISS scenario tree model [1] and demonstrate how the model is applied to help to make design adjustments in CanNAISS.

We describe the CanNAISS model using the standardized format [2]. (1) definitions for the model objective were: the case definition (CD) was NAI virus detected in poultry by virus isolation or PCR; the time period (TP) was 1 month; the design prevalences (DP) were 1% farms and 30% poultry in infected farms; the reference populations were: 685, 2 050, and 2 433 poultry farms for BC Ontario and East&West respectively; (2) initial time period: the first TP was August 2008; the prior probability of infection (at the start of August 2008) was pert(0.49,0.5,0.51); the prior probability of infection (after an outbreak had been resolved) was pert(0.49,0.5,0.51); (3) input: the probability of introduction was pert (0.067, 0.083, 0.1) scaled to the subpopulations: BC, ON, East&West; diagnostic test sensitivities (se) were seVI= pert(0.800, 0.905, 0.990); sePCR= pert(0.750, 0.839, 0.950); seELISA= pert(0.720, 0.889, 0.999); seAGID= pert(0.400, 0.778, 0.999); and seHI=pert(0.800, 0.905, 0.999); (4) data: the number of farms tested were 556, 654 and 1 121 for BC, Ontario and East & West, respectively. The study period was August 2008 to December 2011; (5) model setting: stochastic 1 000 iterations in Excel with PopTools as an add-on; (6) the output was graphical presentations of the posterior probability of each subpopulation being free from NAI at the level of the DP; and (7) validation of the model was performed by the peer review process [1].

We give examples on how the CanNAISS scenario tree model is applied in the design and planning of CanNAISS - for example to allocate sample sizes to the subpopulations and forecast the probability of freedom from NAI

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A Benefit/Cost Financial Management Spreadsheet for Veterinary Laboratory Emergency Preparedness

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Veterinary diagnostic laboratories play a pivotal role in the early and rapid detection of infectious animal diseases that cause serious illness in animals and people, interrupt food supply chains, and negatively impact the economic viability of livestock industries. Most Canadian laboratories have little experience in managing the sudden and exponential increase in testing demand during a foreign animal disease (FAD) outbreak. This type of emergency event causes many logistical challenges for both operational and human resource management. The ability of the laboratory to meet stakeholders' requirements for rapid and accurate diagnostic testing may become strained under these circumstances. Failure to prepare adequately for these contingencies will slow the response to the outbreak, and may negatively impact business continuity.

The objective of this study was to develop a financial template to assist laboratory managers in calculating the various fixed and variable costs associated with emergency laboratory management. A simple Excel spreadsheet includes three main sections:

a) fixed costs associated with emergency planning;
b) variable costs incurred when testing for presence/absence of a foreign animal disease, commonly referred to as surge capacity; and
c) variable costs and lost profit related to business continuity decisions during the outbreak.

A management summary provides a summarized statement of assumptions and costs derived from the three main financial sections.

Laboratory managers can use this spreadsheet to estimate the costs of FAD testing, in addition to costs associated with various business continuity/discontinuity strategies that influence laboratory revenues. These costs are summarized to derive the total financial impact of FAD testing on laboratory operations over a range of outbreak scenarios. Intangible costs such as loss of client goodwill and reduced passive disease surveillance are also highlighted. It is expected that laboratory managers and funding agencies will use this financial management tool to inform their decision-making process, and to support preparations for successful management of FAD testing, thereby ensuring good outcomes for producers, livestock industries and animal health agencies that use veterinary diagnostic laboratory services.


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Determining the gold standard ante-mortem sample type for detection of Brachyspira in pigs

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Brachyspira species can cause colitis and diarrhea in grow-finish pigs. It is a worldwide concern, resulting in great economic losses. Disease control is based on biosecurity measures, and treatment of affected and subclinical pigs. Ante-mortem detection of Brachyspira plays a key role in these processes. Feces are the standard sample, used for culture on selective media. However, these samples are laborious to collect when a large sample number is required, they require refrigeration during shipping for preservation, and samples need to be processed within a limited time. The aim of this study was to determine the sensitivity and specificity of real-time PCR on DNA extracted from feces and rectal swabs compared to fecal culture. A forensic swab requiring no refrigeration (Genotube, Prionics, Switzerland) was used. Samples were collected from 12 pigs pre- and post-inoculation with "Brachyspira hampsonii" strain 30599. During the experiment, fecal culture and fecal consistency score was performed daily, feces and swabs were collected (n=142) every other day. Six pigs with a range of fecal consistency scores were targeted for sampling each day to ensure inclusion of animals with and without clinical signs, ranging from normal feces to mucohaemorrhagic diarrhea. To mimic a real life situation where diagnostic samples are "shipped" overnight to the laboratory, feces and swabs were held for 24h prior to testing. A commercial kit was used to recover total genomic DNA from swabs and fecal samples and real-time PCR for "Brachyspira hampsonii" performed. Real-time PCR data from which collection method was compared to culture results using Fisher's exact test, to evaluate each test's ability to detect the organism. Species-specific real-time PCR on Genotube samples had similar sensitivity to real-time PCR on feces from pigs with both low and high fecal scores, and both compared favourably to culture. Specificity was uniformly high for real-time PCR on both Genotube and fecal samples. These results demonstrate the potential of using Genotube swabs as an epidemiological surveillance tool, due to the ease of sample collection and transport and quality of diagnostic results.

Identification, Molecular Characterization and Electron Microscopy of a new Hemoplasma in Canadian Swine Herd and Development of a Real-Time PCR assay for the Diagnosis of the Infections.

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

Development of an indirect enzyme-linked immunosorbent assay for detection of serum antibodies to leporid herpesvirus-4.

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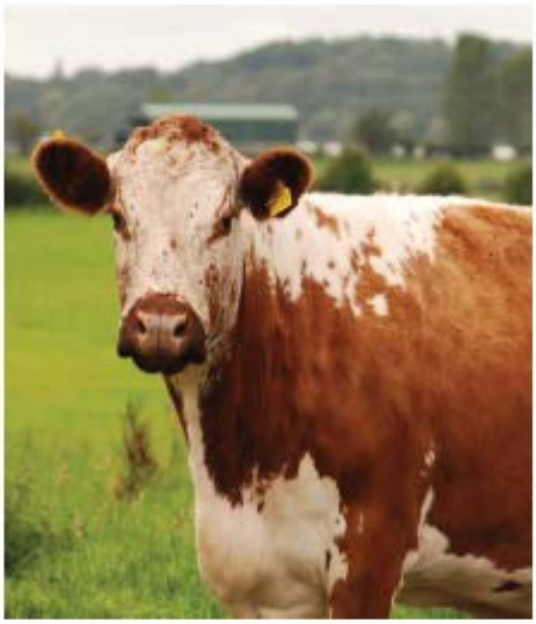
We are developing an indirect enzyme-linked immunosorbent assay (ELISA) test for the diagnosis of leporid herpesvirus-4 (LHV-4), a novel alphaherpesvirus that infects domestic rabbits (Oryctolagus cuniculus). This virus causes severe nonsuppurative pneumonia with high mortality in natural infections and has the potential to have a major economic impact on commercial meat rabbit operations in Canada, making a rapid screening test essential. Our goal is to produce a diagnostic test that is easy to use, sensitive, and to enhance personnel safety, does not require propagation of live virus.

Neutralizing antibodies are produced approximately 10 days following infection with LHV-4 at titres of 1:256 to 1:1024. Polyclonal antibodies produced in rabbits by exposure to adjuvant and inactivated virus have weaker neutralization activity at 1:32 titre dilutions. An indirect enzyme-linked immunosorbent assay (ELISA) was developed using concentrated whole virus absorbed onto a microtiter plate as antigen. Sample:positive (S:P) ratios were calculated and a cut-off value of 0.067 was determined based on 2 standard deviations above readings from 35 negative samples. This was confirmed by virus neutralization (VN) assay. With this cut-off, the test has a sensitivity of 100% and specificity of 95%. S:P ratios for the polyclonal antibodies produced using killed virus are consistently much higher than ratios obtained from sera from infected rabbits.

To develop a sub-unit indirect ELISA assay, we are investigating which antigens are most immunoreactive. Western blot assay has demonstrated the presence of 4 target proteins with apparent molecular masses of 115, 82, 55 and 38 kDa. Both sera from infected rabbits and polyclonal antibodies show similar patterns, but the strength of reaction is much weaker for polyclonal antibodies produced using killed virus, particularly for the 55 and 38 kDa proteins. We hypothesize that the 55 kDa and 115 kDa proteins correspond to glycoprotein D and glycoprotein G, and are in the process of expressing these proteins in a baculovirus vector.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Detection of *Actinobacillus pleuropneumoniae* (App) serotype 1 in pigs by real-time quantitative PCR.

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App is the etiological agent of swine pleuropneumonia. Although it remains a very important clinical problem in many countries, clinical disease is relatively well controlled in USA/Canada. In these two countries, money and efforts are mainly directed toward monitoring sub-clinical infections and keeping herds free of the most common virulent App serotypes. The standardization and application of new diagnostic tools have a tremendous impact on the diagnosis and control/elimination of these sub-clinical infections. Serology is routinely used; however, in a few cases, unexpected positive reactions are observed. In these cases, and especially for breeding herds, confirmation of the presence of the pathogen in tonsils of carrier animals is used to confirm the infection. Isolation of App from tonsils is tedious, time-consuming and a poor sensitive technique. In the present study, two quantitative real-time PCR (qRT-PCR) tests were developed and tested for direct detection of App serotype 1 (CPS1, primers designed for the specific capsular polysaccharide biosynthesis) or App 1-9-11 (LPS1, primers designed for the LPS-O antigen biosynthesis region). Their ability to detect serotype 1 strains as well as their specificity were determined using pure cultures of field and reference strains of different serotypes of App and other related bacterial species genetically close to App and/or susceptible to be present in swine tonsils, such as Actinobacillus suis, Actinobacillus procitonsillarum, Actinobacillus minor, Haemophilus parasuis, Actinobacillus equilii, Actinobacillus lignieresii and Actinobacillus porcinus. For direct detection in tonsils, the analytical sensitivity was evaluated using tonsils experimentally infected in vitro with App serotype 1. Tonsils negative for App were used to evaluate the specificity of both qRT-PCR tests. As expected, qRT-PCR CPS1 reacted with all field strains tested of App serotype 1, whereas that of LPS1 showed reaction with strains of serotypes 1, 9 and 11. None of other strains belonging to different serotypes of App or other bacterial species presented positive reaction with the developed tests. App-negative tonsils were also negative with both tests. The analytical sensitivity of CPS1 and LPS1 PCR were at least 10E5 and 10E4 CFU/0,1 g of tonsil, respectively. Validation of both tests in the field is underway. CPS1 and LPS1 qRT-PCR tests might be useful tools to be used in epidemiological surveillance of App serotype 1. qRT-PCR LPS1 will also be useful for surveillance in European countries, where serotypes 9 and 11 are present.

Caractérisation moléculaire des *E. coli* d'origine utérine chez la vache laitière postpartum

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Après le vêlage, 30% à 40% des vaches démontrent une contamination par *E. coli* dans l'utérus, cependant, seulement 20% d'entre elles présentent les signes cliniques de la métrite par la suite. La présente étude a pour objectif de déterminer un répertoire des gènes de virulence et les groupes phylogénétiques des *E. coli* intra-utérin, associés au développement ultérieur de la métrite chez la vache postpartum. Des écouvillons utérins ont été prélevés sur 497 vaches de race Holstein réparties dans 5 troupeaux, durant la première semaine postpartum et soumis au laboratoire pour identifier les isolats d' *E. coli*. Un total de 258 vaches (52%) ont été positifs à E. coli. et 762 isolats de E. coli ont été isolés et testés pour 40 gènes de virulence représentant les pathotypes ExPEC, STEC, EPEC et ETEC, par la méthode d'hybridation sur colonie. L'étude a révélé 214 combinaisons différentes de gènes de virulence. La plupart des *E. coli* intra-utérins possèdent un ou plusieurs gènes ExPEC dont fimH (95%), HlyE (97%), et iss (70%), sont les plus prévalent. Parmi les autres gènes ExPEC on retrouve sitA (23%), fepC (20%) hra1 (20%) malX (14%) tsh (11%) et bien d'autres. Les gènes non-ExPEC se sont révélés très rare excepté East1(15%). Des isolats possédant des gènes ETEC, STEC ou EPEC n'ont pas, ou ont été très peu été identifiés. Le nombre de gènes de virulence présent dans un isolat varie de 1 à 15. Le groupe phylogénétique le plus prévalent est le groupe B1 (65%) suivis du groupe D (18%) du groupe A (12%) et du groupe B2 (2%). Ces résultats démontrent que certains gènes de virulence ExPEC sont les plus fréquemment observés dans l'utérus de la vache pendant la première semaine postpartum. Les vaches échantillonnées ont été suivies durant la 2ème et 3ème semaine après le vêlage pour diagnostiquer les vaches présentant les signes cliniques de métrite. L'association entre la présence des gènes de virulence et la présence de la métrite est en cours d'analyse.

Development of a new microarray-based diagnostic tool to detect novel viral pathogens involved in potentially devastating animal diseases

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Emerging and re-emerging viral diseases represent a constant threat for animal industry. Current diagnostic methods, such as PCR and ELISA, are known as powerful tools to identify viruses. However, these methods are hindered by limitations such as a high specificity to a narrowed set of viruses. So, in order to select the proper detection method, an a priori knowledge of the virus pathogen suspected to be implicated in a clinical case is required. Since the pathogen(s) implicated in a new disease is unknown, the pathogen identification can become a long and tedious process. For these reasons, the development of a new method for the identification of novel viruses is needed. In this aspect, the development of a DNA microarray is underway in our laboratory. This new DNA microarray is built with highly conserved DNA sequences selected within each important virus family. With this technology, it is hoped that strains from each targeted virus family will be detectable. A year ago, a preliminary DNA-chip has been developed in our laboratory with 200 different probes, targeting 6 different swine viruses (porcine respiratory coronavirus, transmissible grastroenteritis virus, swine influenza virus, porcine reproductive and respiratory syndrome virus [PRRSV], porcine parvovirus and porcine circovirus [PCV]). This preliminary DNA-chip was used to validate the amplification and hybridation methods, and to evaluate the specificity of each selected probes. Obtained results indicate a high specificity of our probes to each virus tested (PRRSV, PCV and swine influenza) and validated our probe selection for the next DNA-chip version. Interestingly, an unrelated equine influenza virus was also detected with the DNA chip and showed the presence of an unknown circovirus, suggesting the detection of a new virus. Last December, the development of the DNA-chip 2nd version was completed. This new DNA chip is composed of 3 806 different probes, including 3 790 probes specifically set to detect over 360 known animal viruses distributed in 19 viral families. This diagnostic method should be available in the coming few months, following validation tests with identified as well as unidentified animal viruses. This technology will be a great addition to our diagnostic laboratory and will help us to identify unknown etiologic viral agents.


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Jane Christopher-Hennings, D.M.V., M.S.

Dr. Christopher-Hennings was trained in the U.S., receiving BS and MS Degrees from the University of Wisconsin-Madison and a DVM from the University of Minnesota. She was in private veterinary practice in South Dakota and at South Dakota State University (SDSU) in a post doctoral position, each for 6 years. She is currently on Faculty at SDSU where she is oversees the Molecular Diagnostics testing. During her time at SDSU, starting in 1990, "Mystery Swine Disease" was just identified and she worked with researchers at SDSU and other collaborators at the University of Minnesota in establishing the etiology (PRRSV). She worked on the initial characterization of the virus by performing C-sections for obtaining gnotobiotic pigs to confirm Koch's postulates in identifying the cause of the disease and developed one of the first PCR based tests for describing the pathogenesis and identification of PRRSV in boars and semen.

Porcine Reproductive and Respiratory Syndrome Virus (PRRSV) Diagnostics: Revisited

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To control PRRSV, various voluntary regional control programs have been started and are on-going. Over the 23 year history of PRRSV diagnostics, many new, improved diagnostic techniques have been developed. However, it is important to not just depend on the techniques, but also view them in the context in which they are used for optimal PRRSV control. A timeline of PRRSV infection and duration of persistence of the virus are important to re-visit in evaluating control programs. Various sample types and quality control measures used within the veterinary diagnostic laboratory for optimum results will also be discussed. In addition, the presentation will include information on possibilities for multiplexed diagnostics to detect PRRSV and other pathogens simultaneously. Since the 2013 International PRRSV Symposium will be held in China on May 18-23, information related to “PRRSV diagnostics and control” from that meeting will be shared.

John C. S. Harding, DVM, MSc, Dipl ABVP (Swine Health Management)

After graduating from the Ontario Veterinary College at the University of Guelph in 1988, Dr. Harding worked in a mixed animal practice in Humboldt, Saskatchewan. He has specialized in swine since 1991, and in 1997 established a swine consultancy practice Harding Swine Veterinary Service. In 1997 he also completed a Master of Science degree in Veterinary Medicine, at the University of Minnesota. Dr. Harding has consulted for both small family and large corporate swine operations in Western Canada. He accepted a faculty position in Swine Production Medicine at the Western College of Veterinary Medicine, University of Saskatchewan in 2004, and was promoted to full professor in 2012. His research interests include emerging and re-emerging diseases, including Brachyspira colitis, periweaning failure-to-thrive syndrome (PFTS), reproductive PRRS, and porcine circovirus. In addition, his team is investigating prenatal immunologic programming associated with fetal uterine growth retardation. Dr. Harding is a frequent speaker at both domestic and international swine meetings, was the recipient of the A.D. Leman Science in Practice Award in 1999, the Pfizer Carl J. Norden Distinguished Teaching Award in 2009, and the Pfizer Animal Health Award for Research Excellence in 2010. He was the founding President of the Canadian Association of Swine Veterinarians from 2004-2005, Vice-President of the IPVS 2010 Congress in Vancouver, and is the Scientific Program Chair of the Western Canadian Association of Swine Veterinarians (WCASV). He has reviewed submissions for a number of peer-reviewed veterinary journals and granting agencies. In 2008 he became a Diplomate of the American Board of Veterinary Practitioners' in the Swine Health Management specialty. In his spare time he enjoys outdoor activities including skiing, sailing, canoeing, hiking and woodworking.

Re-emergence and diagnosis of *Brachyspira* colitis in western Canada

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Discovery of "B. hampsonii"

In October 2009, grow-finish pigs with clinical signs indistinguishable from swine dysentery were observed in two commercial barns in Saskatchewan[1, 2]. Samples submitted from a number of diarrheic pigs to the University of Saskatchewan identified high levels of a novel *Brachyspira* species unrelated to *B. hyodysenteriae (Bhyo).* The organism was temporarily named *B.* sp. Sask30446. Concurrently, researchers in Iowa and Minnesota also identified a novel strongly beta-haemolytic *Brachyspira* associated with severe colitis[3, 4] in American pigs. Since these original observations in 2009-2010, this novel species has been provisionally named *"B. hampsonii"*[5], genetically and phenotypically characterized[5, 6], cultured on agar and in broth culture[6], and used to experimentally reproduce mucohaemorrhagic diarrhea and colitis in susceptible pigs[6, 7].

Since 2009, we have tested over 380 samples from ~70 commercial farms in Canada, and detected or isolated one or more species of *Brachyspira* in about 75% of farms and 60% of

samples. A "sample" refers to feces, tissue, or carcasses from farms demonstrating loose, bloody or mucoid diarrhea in nursery and grow-finish stages. The most prevalent species, "*B. hampsonii",* has been detected in 34% of samples. Of these, approximately three quarters are clade 2 (30446), and one-quarter clade 1 (30599). By contrast, *Bhyo* and *B. pilosicoli (Bpilo)*, long known pathogenic species, have been only detected in 11% and 13% of samples, respectively. While only a small number of farms compared to the size of the western Canadian industry are affected by *Brachyspira* associated colitis, the affected farms represent a sizable portion of the industry and some of the most progressive and biosecure herds in Canada. Moreover, there is no clear explanation for how *"B. hampsonii"* entered some these farms, particularly those at the top of genetic pyramids.

Research merged with diagnostics

Our multi-disciplinary team includes a clinician (J. Harding), molecular (J. Hill) and classical (J. Rubin) microbiologists, biochemist/physiologist (M. Loewen), anatomic pathologists (B. O'Connor, S. Detmer), a graduate student and several technicians. We work out of the WCVM Molecular Microbiology Research Laboratory (MMRL) and are research focused. Our interest in diagnostics relates to improving diagnostic methods and understanding the ecology, epidemiology and impact of *Brachyspira* species affecting commercial pigs. We have no intention to directly compete with commercial diagnostic laboratories, but acknowledge we are filling an industry need. *Brachyspira* diagnostics can be slow, tedious and time-consuming depending on the question being asked by the submitting parties. Whereas running species specific PCR on a given sample is straightforward, our team's discovery of *"B. hampsonii"* has demonstrated the swine industry's need for a more comprehensive approach. This requires multiple diagnostic and epidemiologic tools to identify and speciate any *Brachyspira* that may be present in a sample and determine how it relates to species identified in other farms in a given production system or geographic region. While industry stakeholders gain from understanding what *Brachyspira* is present in the sample or farm, our research-oriented diagnostic service generates many research questions including:

- what is out there?
- what is biologically relevant?
- what factors are associated with pathogenicity?
- what are short and long term control strategies?

Diagnostic methodologies and processes in Saskatoon

Our diagnostic processes have and will continue to evolve over time. We (MMRL) collaborate with Prairie Diagnostic Services Inc. (PDS) with sample acquisition and testing. At present, it is rare for a sample to arrive without having prior contact with a herd veterinarian and/or diagnostic lab. As a result, the farm of origin is identified on all submissions enabling confidential tracking of *Brachyspira* species/strains to the farm, production system and region. We encourage clients to submit carcasses or fresh/fixed intestinal tissues enabling a thorough workup including histopathology and special stains. Although this helps to provide insight regarding the biological relevance microbiological

findings, the most common sample we receive is feces collected from a pen floors. Ensuring timely shipment and appropriate packaging has been a challenge, particularly on fecal samples that are submitted directly from the farm. The submitters have been reasonably compliant with supplying fecal consistency scores (4=bloody, 3=mucoid, 2=runny, 1=wet cement, 0=normal), which is useful in interpreting the microbiological findings.

All samples are submitted to PDS and assigned a diagnostic case number. Given that the MMRL is an external research laboratory and test requests vary, it requires considerable effort to steer samples to their appropriate destination. At present, PDS has designated personnel responsible for liaising with the MMRL, PCR and necropsy laboratories as indicated (or not) on the submission form (Table 1).

Table 1. *Brachyspira* assays routinely performed in Saskatoon

PDS Inc.	**Molecular Microbiology Research Lab**
Histopathology & special stains	Culture
PCR: *B. hyodysenteriae* (Song & Hampson, 2009)	PCR: *Brachyspira* spp. (*nox;* Rohde, 2002)
PCR: *B. pilosicoli* (*cpn60*; Hill unpublished)	RT-PCR *"B. hampsonii"*
	(clade 1; Hill, unpublished)
	(clade 2; Rubin, 2013)
	PCR: *B. hyodysenteriae* (*cpn60*; Hill unpublished)
	DNA purification, sequencing (Macrogen, Korea)
	Phylogenic mapping and interpretation

Strengths & Challenges

Our research-oriented diagnostic service grew from a single case in which *B. intermedia* and *B. murdochii* was reported in a routine diagnostic submission from a pig with bloody diarrhea. This highlights one of the challenges of performing "routine" diagnostics in evolving ecological systems; any answer is not necessarily the right answer. We, as researchers, agreed to look into the case because what had been reported did not make sense. The end result was the discovery of *"B. hampsonii"* clade 2 in western Canada.

The system is not without its challenges. Most recently, we have experienced for the first time, unexplained positive reactions on our *Bhyo* PCR assays. The reasons for these are not fully understood at this time. We also have limited human resources and those available are supporting various other research activities. Sample turnaround is sometimes slower than it should be as a result. Finally, sample receiving and flow has also been confusing for both the client and technicians, an issue made more difficult because of the additional information required on research submission forms and incomplete forms submitted by veterinarians and/or producers.

The strengths of our diagnostic service are our close contact with the submitting vets and knowledge of the production systems they serve. We have a database containing tombstone, clinical and microbiologic information from all cases, and a sequence database used to compare the phylogeny of species/strains amongst farms and production systems.

With these databases, we have identified a number of unique isolates detected repeatedly in diarrheic pigs, which are candidates for *in vivo* pathogenicity testing later this year.

Conclusions

In western Canada, *Brachyspira* colitis has re-emerged as an industry relevant production limiting disease affecting the swine industry. *"B. hampsonii"* is the most commonly species identified, but other known *Brachyspira* species including *Bhyo* are periodically identified in clinical samples. We have also identified a number of unique strains/species and have plans to evaluate their pathogenicity *in vivo*. *Brachyspira* diagnostics are challenging due to the continual evolution of the organism, and because speciation does not always correlate with virulence. We, like many others, believe future diagnostic assays will be based on specific virulence targets characteristic of the pathogenic strains. Until that time, *Brachyspira* diagnostics requires an integrated approach involving anatomical pathology, classical and molecular microbiology, linked to knowledge of what is happening in the field. Offering a select number species specific PCR assays will answer only the most basic diagnostic questions.

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Maria Jose Clavijo, DMV

Dr. Clavijo is a PhD candidate from the University of Minnesota's Swine Group. Her research focuses on the epidemiology of *M. hyorhinis* and the development of diagnostic and genotyping tools for this emerging pathogen. She has presented her research in several international meetings. Dr. Clavijo received her degree in veterinary medicine from the Central University in Venezuela in 2009 where she practiced before starting her PhD in 2010. She is advised by Dr. Albert Rovira and Dr. Bob Morrison.

Update on *Mycoplasma hyorhinis* infection in pigs

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Mycoplasma hyorhinis systemic disease

In recent years there has been a renewed interest in *Mycoplasma hyorhinis* as a major cause of polyserositis and arthritis in post-weaning pigs. This bacterium was first described in 1955 and is commonly found in pig farms, attaching to the upper respiratory tract of swine.[1] Although a commensal, *M. hyorhinis* may cause serious systemic disease. The mechanisms that allow this pathogen to reach systemic sites and cause disease are not fully understood; however, it is believed that differences between strains, concomitant infections and certain environmental conditions promote the development of disease. Reported clinical signs include fever, dyspnea, lameness and reluctance to move. The main lesions caused by *M. hyorhinis* include poliserositis and arthritis mainly in pigs of 3-15 weeks of age. These lesions have been extensively reproduced in experimental inoculations. Acute lesions are characterized by pleuritis, fibrinous pericarditis and peritonitis. Chronic lesions are characterized by pleural and pericardial adhesions. In finishing pigs, *M. hyorhinis* is responsible mainly for arthritis, commonly affecting the hind legs. Joints may contain larger amounts of fluid and fibrin, with the synovial membrane thickened and red. Macroscopically and microscopically the lesions are identical from those caused by *Haemophilus parasuis* (Glasser's disease), it is required to perform PCR tests or to attempt bacterial isolation in order to properly diagnose the disease etiology and determine the appropriate antibiotic treatment. Approximately 55% and 12% of poliserositis and arthritis cases received during 2010 at the University of Minnesota Veterinary Diagnostic Laboratory (UMN-VDL) tested *M. hyorhinis* PCR positive for this pathogen.[2] These data suggest the impact of this pathogen on post-weaning morbidity and mortality. The role of *M. hyorhinis* as a cause of pneumonia is not well understood; however, several studies have isolated or detected *M. hyorhinis* more frequently from pneumonic lungs compared to normal lungs. The experimental inoculation of gnotobiotic pigs combined with stress-inducing techniques has resulted in mild pneumonia lesions. Roughly, 45% of pneumonic lungs received at the UMN-VDL tested positive for *M. hyorhinis* by PCR, but it appears that *M. hyorhinis* may play a secondary role in pneumonia cases.

Dynamics of *M. hyorhinis* infection in commercial swine herds

General aspects of infection caused by *M. hyorhinis* have been reported; however, very little research has been generated in the areas of ecology and epidemiology. This information is vital for designing effective monitoring and control protocols. To begin to generate such needed information, our group has developed a real time PCR[3] and has used this tool to understand the colonization pattern and clinical presentation of *M. hyorhinis* in infected swine herds. In a transversal study conducted it was observed that the prevalence of *M. hyorhinis* nasal colonization in two clinically affected herds was low in sows (average 7%) and suckling piglets (8%) and high in nursery pigs (98%). In contrast, in a herd with no *M. hyorhinis* disease, no colonized sows were found and colonization levels in pigs were very low until the last week in the nursery. In the same study we observed an interesting trend suggesting an effect of parity in the probability of sows to be colonized. Younger sows (parities1 and 2) were more likely to be positive than older sows (parity 3 and older).[4] Subsequently, similar trends were observed in a prospective longitudinal study in two commercial swine herds. Prevalence of nasal colonization in sows and suckling piglets was relatively low, compared to the high proportion of nasal colonization post-weaning. Most pigs became rapidly colonized around 3-4 weeks of age. This rapid colonization might be due to comingling of pigs in the nursery, waning of maternal immunity, removal of antibiotic treatments or a combination of all these factors. However, a parity effect was not evident in these two herds. Additionally, since *M. hyorhinis* can be detected in the oropharyngeal surface oral fluid samples were collected. In both studies PCR results in oral fluids appeared to be correlated with those observed from nasal swabs.[5]

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TECAN.

Preparation of viral RNA for veterinary diagnostics

A versatile platform for automating sample preparation for testing virus-based diseases

Screening of animal diseases using ELISA

Process automation helps to control PRRS and other diseases in swine herds

Posters / Affiches

1- Following Campylobacter jejuni colonization with the use of the high resolution melt analysis of the flaA gene.

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Campylobacter jejuni is a major cause of foodborne infections. It is the zoonotic agent responsible for campylobacteriosis, a gastro enteric disease that may evolve to severe autoimmune disorders. Consuming or mishandling contaminated poultry meat products is an important source of human campylobacteriosis. Lowering C.jejuni presence at the farm level would reduce the risk for humans to acquire the pathogen through the food chain. In a recent trial, a microencapsulated blend of organic acids and essential oils was used in order to reduce the birds Campylobacter caecal load at slaughter. The blend was tested on 2 strains competing for intestinal colonization. Unexpectedly, the C.jejuni caecal level of the treated birds was 1.4 log higher than the control birds. To quickly identify what strains was present in the bird's caecum, the high resolution melt analysis (HRM) of C.jejuni flaA gene was realized, using as template the total DNA extracted from the chicken caecal content. The HRM analysis was done using an Illumina Eco QPCR with previously published C.jejuni flaA primers designed for HRM. DNA from 14 control birds and 12 treated birds was analyzed. Birds were 35 days old and had been simultaneously inoculated at 14 days of age with 2 well characterized C.jejuni strains. HRM showed that 3 C. jejuni HRM-flaA profiles were present in the bird's feces. Similar HRM-flaA profiles could be obtained by using DNA extracted from the inoculated strains. These results are suggesting that the feed additive positively selected for a C.jejuni strain. This is the first study to report the use of C. jejuni HRM-flaA analysis directly in chicken feces.

2- Production and Characterization of Hen Egg Yolk Immunoglobulin Against *Campylobacter jejuni*

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Campylobacter jejuni is the most common bacterial cause of food-borne diseases worldwide. Campylobacteriosis is a self-limiting enteritis that may lead to more severe autoimmune disorders, such as the Guillain-Barré syndrome. Consumption and mishandling of contaminated poultry meat products are the main sources of campylobacteriosis for humans. *C. jejuni* can be considered as commensal bacteria in poultry, colonized birds remain asymptomatic even with 10^9 *C. jejuni* colony forming units (cfu) per gram of intestinal content. *C. jejuni* is detected in chickens often after the 2nd or 3rd week of age, which suggests the presence of natural barriers against *C .jejuni*, like the transfer of maternal immunity (IgY).

The objectives of this study are to obtain a thorough characterization of the presence and specificity of antibodies against *C. jejuni* in egg yolk and to evaluate different protocols to produce IgY antibodies.

To do so, 40 SPF laying hens were separated in 4 groups: gg The inoculations of the hens were done with the same mix of four well characterized strains of *C. jejuni* and were performed at 16 weeks of age. Immunization boosters were given at 21 and 30 weeks of age. Eggs were collected from the beginning of the lay and the IgYs were extracted from egg yolk using a chloroform-based method. Total IgY as well as anti-*C. jejuni* antibody level were determined with an ELISA method. Immuno blots against total proteins of *C. jejuni* strains were performed to compare the specificity of antibodies obtained from each antibody production methods.

Those upcoming results will allow to choose the best method for antibody production to obtain an egg powder against *C. jejuni* that could be used as a feed additive at the farm level to control *C. jejuni* intestinal chicken colonization.

3- Identification of a novel herpesvirus associated with a beluga whale penile proliferative lesion

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An adult male beluga whale (Delphinapterus leucas) was found stranded on the shore of the St. Lawrence Estuary (Rivière-Ouelle, Quebec) on the morning of May 3rd 2008. The carcass was transported on the same day to the "Faculté de médicine vétérinaire de l'Université de Montréal" (FVM- UdM, St. Hyacinthe) for post-mortem examination. Results of this examination suggest that this stranding could be attributed to aspiration pneumonia. Incidental findings included the presence of a well-defined circular raised area (3 X 4 cm) on the penile mucosa. This area grossly described as a thickening of the mucosal layer. Due to the similarity between this lesion and herpesvirus-associated lesions described in dolphins, frozen tissue was sent to the UdM-FVM molecular diagnostic laboratory (MDL) for the detection of herpesviral DNA using a pan-herpesvirus nested polymerase chain reaction (nPCR) diagnostic assay (following standard DNA extraction). The pan-herpesvirus nPCR is known to be able to detect a broad range of herpesvirus species and target specifically the DPOL gene of the viral genomes. The pan-herpesvirus nPCR assay was found positive, and the amplified DNA was sequenced. Overall, the DNA sequence of the obtained PCR product (208 nucleotides in length) suggests the existence of a new Alphaherpesvirinae since its highest similarity was associated with different alphaherpesviruses. It's highest sequence homology was with cercopithecine herpesvirus (82,5% homology), baboon herpesvirus (82,5%), human herpesvirus 1 (84,4%) and dolphin herpesvirus (79,3%). Other sea mammals herpesviruses were found to be genetically similar such as blainville's beaked whale gammaherpesvirus (79,8% homology), delphinid herpesvirus 8 (78,2%), false killer whale alphaherpesvirus (77,9%) and tursiops trucatus alphaherpesvirus 2 (74,2%). Studies are in progress to characterize this new beluga whale herpesvirus such as virus isolation, electronic microscopy and high-throughput sequencing.

4- High-throughput sequencing revealed the presence of a recently discovered porcine parvovirus (porcine partetravirus) in Canadian swine population

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In the course of a porcine reproductive and respiratory virus (PRRSV) full genome sequencing research project, 62 swine sera samples collected in farms from across Canada were submitted to the laboratory for analysis. PRRSV viral particles were purified and RNA was extracted. In order to increase the number of PRRSV DNA fragments, a random PCR amplification protocol was used. Afterward PRRSV genome quantification was established by qPCR and 11 samples with high amount of PRRSV genome were subsequently selected and sent to the "Institut de biologie integrative et des systems" (IBIS) for high-throughput sequencing. Following data analysis, a contaminant DNA sequence related to a new parvovirus, recently reported in swine population, was detected in one of the 11 submitted samples (9% of all samples). The parvovirus positive sample was obtained from an Ontario pig farm. The obtained DNA sequence was highly similar to the porcine partetravirus (also known as porcine hokovirus, porcine PARV4-like virus or porcine parvovirus 3) and has been detected in multiple countries, including the United States, China, Great-Britain, Romania and Germania. To our knowledge, the porcine partetravirus has never been reported in Canada. The identified partetravirus genome length is 5402 nucleotides and was assembled from 938 reads with a mean coverage of 48 reads per base (with a minimum of 8 reads and a maximum of 85 reads). Unfortunately, to our knowledge, the porcine partetravirus has not been isolated in cell culture so far. Porcine partetravirus has not been found to be an etiological agent of diseases and thus, seems to be non-pathogenic. Nonetheless, Dr Opriessnig's research team reveal that the prevalence of partetravirus has been at its highest level in pigs suffering from enteric disease (2,7% of all cases), with systemic/central nervous system disease manifestation (11,6%) or with respiratory disease (14,4%). These results could suggest that the porcine partetravirus might be associated with those symptoms but it still need to be proven. In addition, the virus was detected mostly in adult pigs and nursery pigs, and was not detected in fetuses or suckling pigs. Further studies will be needed to assess the porcine partetravirus prevalence within the Canadian swine population and its potential involvement in clinical diseases.

5- Antimicrobial resistant profile and virulence factors of Escherichia coli isolates from San Pedro River in Aguascalientes, Mexico.

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Surface water pollution in developing countries is an important health concern and may represent a significant mechanism of dissemination of pathogenic bacteria and antibiotic resistance genes. In the present study, 150 Escherichia coli isolates were collected from San Pedro River in Aguascalientes, Mexico. Clinical Laboratory Standards Institute disk diffusion method was used to determine resistance phenotype to 13 different antibiotics, including the fluoroquinolones levofloxacine and pefloxacine. Thirty percent (46/150 isolates) of the isolates exhibited multidrug-resistant phenotype. Eighteen strains were quinolone resistant, of them, 16 showed multi-resistant patterns. Most of the isolates were classified by PCR as commensal (n=59), but virulence genes associated with enteropathogenic (n=10), enterotoxigenic (n=9), enteroaggregative (n=67) and extraintestinal (n=5) E. coli were also detected in some isolates. An E. coli specific microarray (Maxivirulence V 3.1) was used to characterize the virulence and antimicrobial resistance genes profiles of the fluoroquinolone-resistant isolates. Furthermore, the presence of mutations conferring resistance to quinolone in gyrA and parC genes, and the presence of plasmid-media quinolone resistance (PMQR) genes qnrA, qnrB, qnrS and aac-(6')-lb-cr were investigated. The occurrence of PMQR genes was detected in 12 isolates. Other antimicrobial resistance genes such as blaTEM, sulI, sulII, dhfrIX, aph3(strA) and tet(B) were also found. Single or double mutations at Ser-83→Leu, Asp-87→Asn in the gyrA gene were detected in 66.6% (12/18) of the isolates. Mutation at Ser-80→Ile in parC gene was detected in 50.5% (9/18) of isolates. One isolate carrying human enterotoxigenic genes presented a multi-drug resistance phenotype and genotype. Moreover, the isolate not only possesses two fluoroquinolone acquired-genes (aac-(6')-lb-cr, qnrS), but also triple chromosomal mutations (Ser-83→Leu, Asp-87→Asn in gyrA, and, Ser-80→Ile in parC). These results show that potential pathogenic and antibiotic resistant E. coli are present in the San Pedro River and these E. coli could be a potential threat to human and animal health.

6- Identification and distribution of an ETEC: F4 contamination marker in a swine production network.

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A number of studies have demonstrated a link between the detection of potentially pathogenic Escherichia coli strains and economic loss in the swine industry. The transmission of such E. coli between herds and the slaughterhouse and vice versa, in the network they constitute, has not been well characterized. A better understanding of the movement of these E. coli between the different stages of food production could lead to better herd health status and contamination control in the slaughter process. E. coli strains belong to different clonal groups which may be commensal or pathogenic, the latter being characterized by the presence of specific virulence genes. Thus, the presence of certain of these genes, as detected by molecular techniques, could be used as an indicator of contamination between herds and the slaughterhouse. The objective of this study was to examine the transmission modes of E. coli in a defined swine production network. A geographical region containing 10 finishing farms, a slaughterhouse, and a transportation network was selected. Periodic samples were collected at various sites on the farms (3 visits), at the slaughterhouse (2 visits), and on the vehicles of stakeholders linking the farms and slaughterhouse, such as animal transporters and veterinarians. Three consecutive production batches were followed during 8 months. The presence of virulence genes (eltB, estA, estB, faeG, stxA, stx2A, eae, cnf, papC, iucD, tsh) most commonly associated with pathogenic E. coli was examined in the samples by conventional multiplex PCR. The monitoring of the virulence gene distribution profiles both temporally and spatially gave us the opportunity to identify an ETEC: F4 marker. The ETEC: F4 distribution suggests the slaughterhouse yards act as a reservoir of contamination in the network. Further characterization and comparison of ETEC: F4 strains present in the network could identify contamination events between the farms and the associated slaughterhouse.

7- Global animal pathogenic and zoonotic Escherichia coli (APZEC) database

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Escherichia coli is an important cause of disease in production animals and humans worldwide. In certain cases, animals are a reservoir of infection for humans. Animal pathogenic and zoonotic *E. coli* (APZEC) are *E. coli* which are pathogenic in animals or which are harmless in the animal reservoir but are potentially pathogenic in humans. To date, it has been difficult to monitor trends in virulence profiles and antimicrobial resistance (AMR) of pathogenic *E. coli* from animals, due to a lack of standardized system for characterization of these isolates. Thus, the objective of this work was to set up a database for the surveillance of virulence gene profiles and AMR of pathogenic *E. coli* in animals.

Over 2,500 *E. coli* isolates from all production and companion animal species, mostly from clinical cases of pigs, cattle, and poultry, that have been submitted to the Diagnostic Service of the Faculté de médecine vétérinaire de l'Université de Montréal between 2008 and 2012, were examined. These isolates were examined by PCR and colony hybridization for the detection of the presence of genes coding for the 20 virulence factors most frequently found in pathogenic *E. coli* in animals. AMR was also determined by the standard disk diffusion assay by Ministère de l'Agriculture, des Pêcheries et de l'Alimentation du Québec. AMR, demographic and clinical data were gathered resulting in an integrated profile.

Data are accessible on a web platform (www.apzec.ca), as interactive drill down and multi-series charts. Preset multi-series charts have been prepared to show the most frequently observed trends. In addition, quarterly reports showing trends in prevalence of cases involving clinically important patho-virotypes or in resistance to antimicrobials will also be available on the web platform.

The database serves as an indicator of animal health and as a tool for monitoring spatio-temporal trends in virulence and antimicrobial resistance in APZEC. More specifically, it will provide guidance in the setting up of appropriate strategies for the diagnosis and control of infection in animals on-farm and in the evaluation of the effectiveness of these strategies. Thus, the database will contribute to improving animal health, the adoption of a more judicious use of antimicrobials in animals, and to reducing the danger to human health due to transfer of APZEC and AMR genes.

8- Primary anaplastic leiomyosarcoma in the mandible of a rescued Virginia Opossum (Didelphis virginiana)

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An approximately 2-year old intact female rescued Virginia Opossum (Didelphis virginiana) was presented for recurrence of a right mandibular mass. Previous surgical excision and histopathology of this mass afforded a diagnosis of an anaplastic sarcoma most consistent with osteosarcoma. Radiographs showed aggressive bone lysis of the right mandible with loss of associated teeth which supported this diagnosis. Given the scarcity of knowledge about neoplasia in this species and the projected poor quality of life, the keepers elected for humane euthanasia. At necropsy the caudal 50 % of the right mandibular ramus was effaced by a firm, well demarcated, multilobular, 6 x 4 x 3.5 cm, mottled white, tan, red and brown necrotic mass. There was marked lymphadenopathy of both the right mandibular and superficial cervical lymph nodes, both of which were diffusely effaced by the same firm nodules. Histologically the mass was composed of haphazard streams, bundles and dense sheets of large, often individualized, plump round anaplastic spindle-shaped cells with large amounts of finely granular eosinophilic cytoplasm and large round to oval nuclei. Rare scattered clusters of elongate spindle-shaped cells, measuring approximately 40 x 15 um, with variably distinct angular to rectangular cell membranes, markedly expanded granular to subtly fibrillar eosinophilic cytoplasm and occasional linearly arranged elongate oval to rod shaped nuclei were scattered throughout the mass. There was no evidence of osteoid matrix production and the presence of elongate cells and linear nuclear orientation together with subtle fibrillar appearance of the cytoplasm was highly suggestive of a muscular (smooth or striated) origin for this mass. No cross striations were observed with a phosphotungstic acid haematoxylin (PTAH) stain. Immunohistochemistry (IHC) was performed in order to determine the expression of vimentin, S100, MHC II, MAC387, desmin, pan-actin and α-smooth muscle actin to further evaluate the mesenchymal phenotype of this neoplasm. Neoplastic cells were immunoreactive with antibodies against α-smooth muscle actin, pan-actin and vimentin but not with S100, MHC II, MAC387 and desmin antibodies. This staining pattern is consistent with a mesenchymal tumour of smooth muscle origin and a final diagnosis of leiomyosarcoma was afforded. This is the first report of an anaplastic leiomyosarcoma at any site in a Virginia Opossum and adds to a small number of reports describing neoplasms and immunohistochemical procedures in this species. Within the veterinary literature, this case is atypical, in so far as it is the first leiomyosarcoma to have presented as a primary mandibular mass.

9- Effect of colistin sulfate on fecal Escherichia coli and state health of weaned pigs challenged with Escherichia coli F4 (K88)

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Colistin sulfate (CS), a peptide antibiotic, is recommended for the therapy of gastrointestinal tract infections in pigs. The target of antimicrobial activity of CS is the bacterial cell membrane. Colistin sulfate displaces magnesium (Mg+2) and calcium (Ca+2), which normally stabilize the LPS molecules, from the negatively charged LPS, leading to leakage of intracellular contents and, subsequently, cell death. This antibiotic is used off label in Canada for the treatment of post-weaning diarrhea. The aim of the present study was to evaluate the efficacy of CS in the treatment of post-weaning diarrhea and its effect on the evolution of Escherichia coli population. A total of 14 weaned piglets were used. At 28 days of age, pigs were challenged orally with 1×109 cfu of an ETEC F4 resistant to naladixic acid. Pigs were divided into two groups, control group (n=7) and a treated group (n=7) received a single oral dose of CS at 50,000 IU/Kg, given 3 days after inoculation. Severity of diarrhea was monitored using a fecal consistency scoring system. Bacterial counts was carried out by streaking faecal samples on to Petrifilm E. coli/Coliform count plates, and 5 per cent bovine blood agar plates, containing nalidixic acid, for detection of ETEC F4 strain. Faecal samples were collected before challenge and 24, 72, 84, 96, 108, 120 h post challenge. A necropsy was performed 120 h post challenge and ileal mucosa was collected to evaluate bacterial colonization. Level of dehydration was determined by measuring total protein (TP). In both groups, there was an increase in bacterial counts following challenge. After CS treatment, especially at 24 h post treatment there was a decrease in bacterial count as compared to the control group. The consistency, ETEC F4 attachment to the ileal mucosa and TP concentrations were not affected by antibiotic treatment.

In this pilot study, administration of a single dose of CS resulted in a reduced bacterial count of E. coli population and ETEC F4. However, scores of diarrhea, attachment of ETEC F4 to the ileal mucosa and dehydration were not affected by CS treatment. Further investigations with a complete therapeutic protocol and a quantitative real-time PCR are needed, to evaluate effect of CS on ETEC F4 diarrhea, total E.coli populations and on the intestinal microbiome.

10- Evaluation of feeding strategies to reduce Salmonella infection in piglets

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In order to minimize incidence of diseases caused by different pathogens, a combination of functional feed additives capable of modulating the development of systemic and mucosal immune system barrier function of intestinal wall and bacterial populations in the gut were examined. This first part of the project was to determine the proper dose of Salmonella Typhimurium needed to establish the carrier state in piglets. Three groups of 10 piglets have been exposed to different oral doses of S. Typhimurium and the group 4 was the control group (n=5). A clinical exam was done to each piglet twice a day. Fecal samples were taken and analysed to evaluate Salmonella shedding post-infection. Piglets were euthanatized at different days and mesenteric lymph nodes were taken to detect and quantify Salmonella. The results demonstrated that piglets receiving the higher dose (10X8 CFU/mL) showed the highest level of carrier state during the Salmonella challenge and this dose has been used for the infection model. The second specific objective of this project was to evaluate the impact of functional food on the course of S. Typhimurium infection in piglets. Piglets were assigned to 1 of the 4 treatments as follow: 1- control (CTRL), 2- antibiotic (ATB), 3- cocktail of functional food (CFF), 4- bovine colostrum + cocktail of functional food (COL-CFF). At 49 days of age, piglets were orally inoculated with S. Typhimurium, and each piglet was followed clinically. Fecal samples were taken to evaluate post-infection Salmonella shedding. Two piglets per litter have been euthanized at 2 different periods. Ileo-ceacal junction and mesenteric lymph nodes were taken to detect and quantify Salmonella. Regarding clinical signs, the CTRL group showed higher fecal consistency scores 3 days post-infection. Piglets in group ATB excreted lower Salmonella levels than piglets in the CTRL group. Groups receiving the CFF diet and the COL-CFF diet showed lower fecal consistency scores then CTRL group.

11- Diagnosis of swine respiratory infectious agents using a nano-volume quantitative PCR platform.

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Objectives: To develop a novel diagnostic approach for simultaneous identification and quantification of swine respiratory infectious agents bringing losses to the swine industry. We will evaluate the OpenArray® technology from Life Technologies, a platform based on nanofluidic q-PCR technology using a specific design of primers and fluorophore-based probes.

Validation of primers and probes: Primers and probe targeting specific viral and bacterial genes were selected using bioinformatics. For each sets, sensitivity, specificity and reaction efficiency (90% > E > 110%) were first evaluated by qPCR on RNA and DNA targets. Standard dilutions and specificity panels were run on a SmartCycler Real-Time PCR system (Cepheid) and only the acceptable assays were included in our custom-made OpenArray.

Openarray assays: A first group of preliminary tests on our panel of reference and clinical isolates has been performed successfully using our custom OpenArray assays on the recently installed QuantStudio® Platform. All assays were able to reproductively detect their target genes in the cognate isolates, and detected several different targets in some clinical samples.

Bacterial detection: All 7 of our current bacterial targets (including A. pleuropneumoniae, and S. suis) were detected, with a threshold similar to the Cepheid's SmartCycler. Tests targeting M. hyorhinis and hyopneumoniae both demonstrated 100% specificity and sensitivities of 88 and 75% respectively.

Virus detection: The PRRSV-NA assay allowed detection of diverse isolates such as Klop and Newport, and did not detect the European variant. The limit of detection was comparable to the Smartcycler. The PCV2 assay targeting subtypes 2a and 2b also proved high specificity and a detection threshold below 10^5 copies/rx. The influenza assays targeting the matrix gene was able to detect cDNA from H1N1 and H3N2 clinical isolates. The assays specifically targeting the H1 or H3 and N1 or N2 genes variants were specific and sensitive.

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