PROCEEDING OF THE

2019

18th Annual Meeting of the CAHLN // 18e Réunion annuelle du RCTLSA

« The last frontier in veterinary diagnostic; applied genomic and high throughput sequencing »

« La dernière frontière en diagnostic vétérinaire; la génomique appliquée et le séquençage à haut débit »

Hosted by/ Reçu par la

Faculté de médecine vétérinaire
Université de Montréal
Saint-Hyacinthe
Québec

Canadian Animal Health Laboratorians Network

Réseau canadien des travailleurs des laboratoires de santé animale

2018-2019 CAHLN / RCTLSA

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The 2019 CAHLN Meeting is made possible by:

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Table of content // Table des matières

18th ANNUAL MEETING OF THE
CANADIAN ANIMAL HEALTH LABORATORIANS NETWORK
(CAHLN)

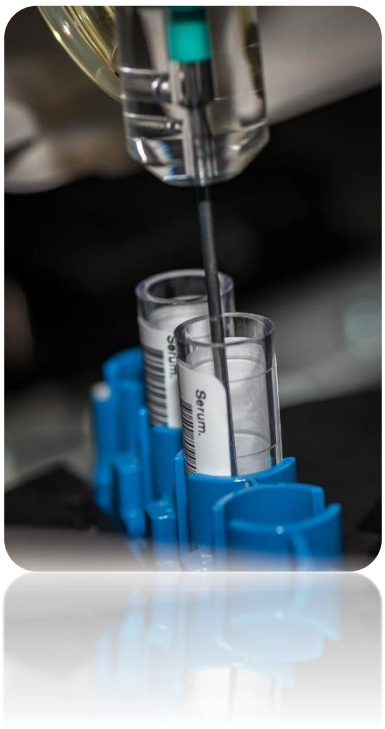
May 26-29, 2019

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.



The 2019 local organizing committee is comprised of:
Estela Maria Cornaglia (Chair)
Véronique Allard
Véronique Boyer
Guy Fontaine
Maria Zardon
Nadine Messier
Louise Bazin

The 2019 Scientific committee is comprised of:
Estela Maria Cornaglia DMV, PhD
Carl Gagnon DMV, PhD
Julie-Hélène Fairbrother DMV, MSc, DACVM
Olivia Labrecque DMV, MSc, DACVM

18e RÉUNION ANNUELLE DU

RÉSEAU CANADIEN DES TRAVAILLEURS DES LABORATOIRES DE SANTÉ ANIMALE (RCTLSA)

26 au 29 mai 2019

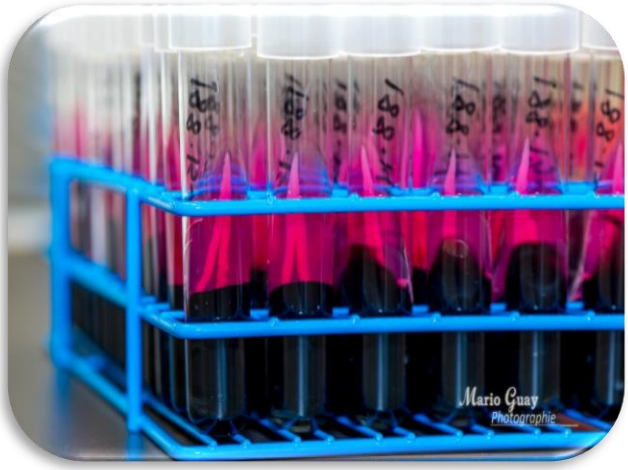
Faculté de médecine vétérinaire de l'Université de Montréal,
Saint-Hyacinthe, Québec

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.

Le comité organisateur de la réunion de 2019 est composé de:
Estela Maria Cornaglia (Chair)
Véronique Allard
Véronique Boyer
Guy Fontaine
Maria Zardon
Nadine Messier
Louise Bazin



Le comité scientifique de la réunion de 2019 est composé de:
Estela Maria Cornaglia DMV, PhD
Carl Gagnon DMV, PhD
Julie-Hélène Fairbrother DMV, MSc, DACVM
Olivia Labrecque DMV, MSc, DACVM

Laboratorian of the Year

Prix du diagnosticien de l'année

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 winners // Anciens lauréats:

2003 - Lloyd Spencer, CFIA, Nepean, ON
2004 - Ian Barker, OVC, Guelph, ON
2005 - Marcelo Gottschalk, FMV, St. Hyacinthe, QC
2006 - John Robinson, MAL, Abbotsford, BC
2007 - John Fairbrother, FMV, St. Hyacinthe, QC
2008 - W.D.G (Bill) Yates, CFIA, Lethbridge, AB
2009 - Gerald R. Johnson, AVC, Charlottetown, PEI
2010 - Ted Clark, Calgary, AB
2011 - Josepha DeLay, AHL,Guelph, ON
2012 - Mark Swendrowski, MAFRI, Winnipeg, MB
2013 - Grant Maxie, U. of Guelph, Guelph, ON
2014 - John Pasick, CFIA, Winnipeg, MB
2015 - James P. Goltz, NBDFA, Fredericton, NB
2016 - Alfonso Lopez, AVC (retired), UPEI, Charlottetown, PEI
2017 - Doug Campbell, Guelph, ON
2018 - Yanyun Huang, PDS, Saskatoon

Graduate Student Presentation Award

Prix pour meilleure présentation remis à un étudiant gradué

A plaque is awarded annually to a graduate student based on the quality of his or her presentation at the CAHLN annual meeting. In addition to the plaque, a cash prize of 400$ sponsored by the CRIPA will be given to each winner. 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. En plus de la plaque, un prix en argent de 400$, commandité par le CRIPA, sera remis à chaque gagnant. 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 winners of Graduate Student Presentation Award //
Anciens gagnants du prix de meilleure présentation remis à un étudiant gradué

2003 – Sherry Andrews, WCVM, Saskatoon, SK
2004 – Noel Harrington, OVC/CFIA, ON
2005 – Guillaume Bruant, FMV, St. Hyacinthe, QC
2006 – Yuanmu Fang, WCVM, Saskatoon, SK
2007 – Kathi Ellis, WCVM, Saskatoon, SK
2008 – Angela Catford, OVC, Guelph, ON
2009 – Raphael Vanderstichel, AVC, Charlottetown, PEI
2010 – Guilherme Gomes Verocai, UCVM, Calgary, AB
2011 – Olivier Côté, OVC, Guelph, ON
2012 – Jason Struthers, WCVM, Saskatoon, SK
2013 – Janet Sunohara-Neilson, OVC, Guelph, ON
2014 – Cathy Bauman, OVC, Guelph, ON
2015 – Oral: Arinjay Banerjee, Dept. of Veterinary Microbiology, Univ of Sask
Poster: Thushari Gunawardana, Dept. of Veterinary Pathology, Univ of Sask
2016 – Oral: Christina Solis Worsfold, UCVM, Calgary, AB Poster: Iman Mehdizadh Gohari, OVC, Guelph, ON
2017 - Oral: Jamie Rothenburger Guelph, ON and Ellie Milnes Toronto Zoo, ON
Poster: Corrine Schut Guelph, ON
2018 - Oral: Maodong Zhang, Saskatoon Poster : Samira Yousefi, OVC, Guelph, ON

Graduate Student Travel Awards

Bourse de voyage pour étudiants gradués

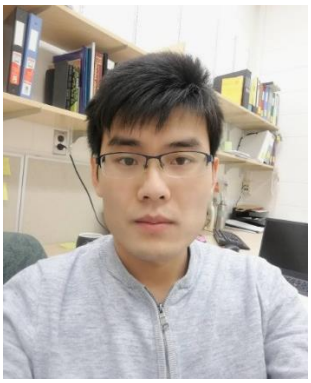
The travel awards are intended to help defray the cost of travel and lodging for students traveling to the Annual CAHLN meeting from sites distant to the host site, encouraging student excellence in their laboratory discipline, developing presentation skills (verbal, written, organizational), and promoting networking of students with veterinary laboratorians. This year, funds are available for 2 travel awards of $750 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 vétérinaires. Cette année, les fonds sont disponibles pour 2 bourses de voyage de 750 $ 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.

2019 STUDENT WINNERS



Maodong Zhang
University of Saskatchewan

Daniel Gibson
University of Guelph

ÉTUDIANTS GAGNANTS 2019

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Thanks to our sponsors! / Merci à nos partenaires!

CAHLN//RCTLSA 2019

Program // Programme

Sunday, May 26, 2019 / Dimanche 26 mai 2019

17:30 – 20:30 **CAHLN Evening Reception** (includes: Registration, Diner Cocktail, Art Exhibit, Guided tour of laboratories)

RCTLSA Réception de bienvenue (inclut : Inscription, Cocktail dînatoire, Exposition d'œuvres d'art et Visite guidée des laboratoires)

CDEVQ
3220 Sicotte, Saint-Hyacinthe,
Quebec J2S 2M2

Monday, May 27, 2019 / Lundi 27 mai 2019

Faculty of Veterinary Medicine

Université de Montréal

3200 Sicotte, Saint-Hyacinthe

Québec (J2S 2M2)

07:30 – 08:15 Registration // Inscription

08:15 – 08:30 Official Opening and Welcome (Room 1134) by // Ouverture officielle et bienvenue (Local 1134) par:
Marie-Claude Bélanger - Vice-Dean, Clinical Affairs and Professional Training //Vice-doyenne aux affaires cliniques et à la formation professionnelle
Estela Cornaglia - Director of the Diagnostic Service// Directrice du Service de diagnostic

Applied genomic and Next Generation Sequencing // Génomique appliquée et séquençage de nouvelle génération

Moderator// Modérateur: Carl Gagnon (Room/Local 1134)

08:30 – 09:15 Yanyun Huang "Adapting high throughput sequencing in an animal health laboratory – where are we and where to go?"

09:15 – 10:00 Durda Slavic "Pioneering a new technology in accredited veterinary diagnostic laboratory - things to look forward to, or not…"

10:00 – 10:30 *Break, posters, exhibits (Room 0135) // Pause, affiches, exposants (Local 0135)*

Moderator// Modérateur: John Morris Fairbrother (Room/Local 1134)

10:30 – 11:15 Carl Gagnon "Day-to-day challenges in a high throughput sequencing diagnostic laboratory"

11:15 – 12:00 Oliver Lung "High throughput sequencing at Canada's Only Containment Level 3 HTS Facility: The first 3 years"

12:00 – 13:30 *Lunch, posters, exhibits (Meet the Authors in front of their poster between 12h30 and 13h30 - Room 0135) // Dîner, affiches, exposants (Rencontrez les auteurs devant leur affiche entre 12h30 et 13h30 - Local 0135)*

12:30 – 13:30 CAHLN Business Meeting (open) (Room 1125) // RCTLSA Réunion d'affaire (ouvert) (Local 1125)

Moderator// Modérateur: Marie Archambault (Room/Local 1134)

13:30 – 14:15 John M. Fairbrother "Development of a whole genome sequencing approach for identification and antimicrobial resistance of pathogenic *Escherichia coli* in the veterinary diagnostic laboratory"

14:15 – 15:00 Discussion panel // Panel de discussion

15:00 – 15:30 *Break, posters, exhibits (Room 0135) // Pause, affiches, exposants* (*Local 0135)*

Moderator// Modérateur: Chantale Provost (Room/Local 1134)

15:30 – 15:50 Chantale Provost "Genetic diversity preliminary results of swine influenza virus in Quebec, Canada, in 2018"

15:50 – 16:20 Christopher Adams (Ion Torrent scientific presentation), Targeted genotyping by sequencing (tGBS) a flexible and economical solution for multiple high throughput genotyping applications

16:20 – 16:50 Kahlil Lawless (Illumina scientific presentation), Illumina: Advances of microarray and NGS in animal breeding and health.

16:50 *Adjourn // Levée des conférences*

17:00 Guided tour of laboratories // Visite guidée des laboratoires

17:00 – 20:00 CAHSN Executive Board meeting (by invitation) (Room 1125) // CAHSN Réunion du conseil exécutif (sur invitation) (Local 1125)

Tuesday, May 28, 2019 / Mardi 28 mai 2019

CAPV special session CAHLN // ACPV session spéciale au RCTLSA

Moderator // Modérateur: Maria Spinato (Room/Local 1134)

08:15 – 08:30 Maria Spinato "Using molecular tools to identify previously undetectable causes of ovine abortion – *Helicobacter trogontum*"

08:30 – 08:50 Isabelle St-Pierre, Review of necropsy cases in Québec

08:50 – 09:10 Isabelle St-Pierre, Interesting cases in Québec

09:10 – 09:30 Magaly Bégin-Pépin, PISAQ Abortion in small ruminants

09:30 – 10:00 Julie-Hélène Fairbrother and Sonia Chénier "Postmortem Laboratory FAD Emergency Exercise - First experience"

10:00 – 10:30 *Break, posters, exhibits (Room 0135) // Pause, affiches, exposants* (*Local 0135)*

10:30 – 10:50 "Provincial Vet. Lab diagnostic reports"

10:50 – 11:20 Marcelo Gottschalk "Diagnosis of *Streptococcus suis* in an affected farm: harder than expected"

11:20 – 11:40 Younes Chorfi "Measuring deoxynivalenol in animals"

11:40 – 12:00 Younes Chorfi "Selenium levels in Quebec horses"

12:00 – 13:30 *Lunch, posters, exhibits (Meet the Authors in front of their poster between 12h30 and 13h30 - Room 0135) // Dîner, affiches, exposants (Rencontrez les auteurs devant leur affiche entre 12h30 et 13h30 - Local 0135)*

12:00 – 13:30 *TSE Meeting (by invitation)* (Room 1125) *// Réunion TSE (sur invitation)* (Local 1125)

Student session

Moderator // Modérateur: Julie-Hélène Fairbrother (Room/Local 1134)

13:30 – 13:45 Kaye Quizon "Development and validation of an indirect ELISA for the serological diagnosis of African swine fever"

13:45 – 14:00 Maodong Zhang “Respiratory viruses identified in western Canadian beef cattle by thigh throughput sequencing and their association with bovine respiratory disease”

14:00 – 14:15 Mahder Teffera “Generation of Capripoxvirus vaccines to differentiate vaccinated animals from unvaccinated animals and validation of a competitive enzyme linked immunosorbent assay to test vaccine efficiency”

14:15 – 14:30 Christian Lalonde “Whole genome sequencing of porcine reproductive and respiratory syndrome virus (PRRSV) from clinical samples”

14:30 – 14:45 Sean Yeo “Detection of foot-and-mouth disease virus in swine meat juice”

14:45 – 15:00 Daniel Gibson “Multiplex real-time PCR assay for three psittacine viruses”

15:00 – 15:30 *Break, posters, exhibits (Room 0135) // Pause, affiches, exposants* (*Local 0135)*

Moderator: Neil Pople (Room 1134)

15:30 – 16:00 Grant Maxie “Evolution of veterinary lab testing – from the trenches”

16:00 – 16:30 Johnny Callahan (Tetracore scientific presentation), Cutting edge technology: direct field testing using a portable instrument without nucleic acid extraction.

16:30 – 16:45 Michael Mulvey “Building a new AMR surveillance system in Canada: AMRNet”

17:00 *Social activity, Bateau Mouche, departure of bus. // Activité sociale, Bateau Mouche, départ de l’autobus*

18:30 – 22:00 *Social activity, Bateau Mouche // Activité sociale, Bateau Mouche*

Wednesday, May 29, 2019 / Mercredi 29 mai 2019

Plenary session // Session plénière

Moderator // Modérateur: Grant Maxie (Room/Local 1134)

08:10 – 08:40 Anatoliy Trokhymchuk "Canadian Animal Health Technology and Intelligence Networks (CAHTIN) overview"

08:40 – 09:00 David W. Silversides "Labgenvet.ca, a bilingual web-based resource for genetic diseases in domestic animals"

09:00 – 09:20 Rémi Froment "Optimizing the immunocytochemistry technique"

09:20 – 09:40 Musangu Ngeleka "Rapid detection of bovine respiratory bacterial pathogens and associated antimicrobial resistance profiles by whole-metagenome sequencing of clinical samples"

09:40 – 10:00 Musangu Ngeleka "Diagnosis of canine urinary tract infection; laboratory versus in-clinic test accuracy to guide antimicrobial selection"

10:00 – 10:30 *Break (Room 0135) // Pause (Local 0135)*

Moderator // Modérateur: Musangu Ngeleka (Room/Local 1134)

10:30 – 10:50 Sonia Lacouture "Distribution of *Streptococcus suis* (from 2015 to 2018), *Actinobacillus pleuropneumoniae* and *Haemophilus parasuis* (from 2018 to April 2019) isolated from diseased pigs in Quebec"

10:50 – 11:10 Darren Boese "An introduction to the Canadian Animal Health Surveillance Network (CAHSN) laboratory support team and the implementation of the African Swine Fever (ASF) proficiency testing program"

11:10 – 11:30 Chantale Provost "Molecular characterization of complete genome of newly emerging avian reovirus variants and novel strains in Quebec, Canada, 2016-2017"

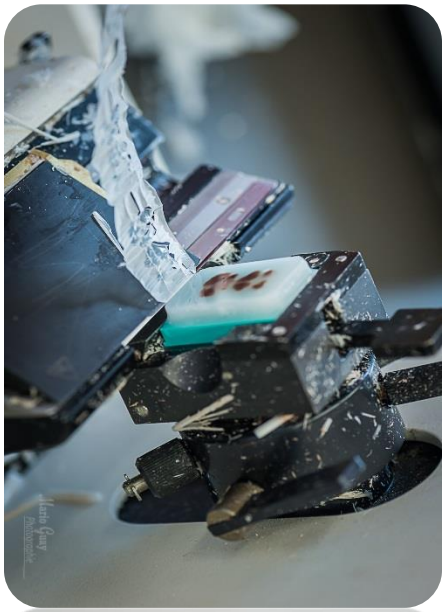
11:30 **Presentation of awards:**

- *CAHLN Graduate Student Oral/Poster Presentation Awards*
- *CAHLN Graduate Student Travel Awards*
- *CAHLN Laboratorian of the Year*

Présentation des prix:

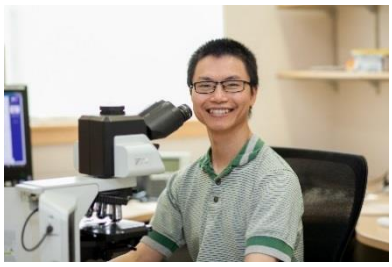
- *RCTLSA Meilleure présentation Orale/Affiche d'un étudiant gradué*
- *RCTLSA Bourse de voyage à deux étudiants gradués*
- *RCTLSA Diagnosticien de l'année*

12:00 *Levée du congrès // Adjourn*



More about our invited Speakers //

Plus sur nos conférenciers invités



Yanyun Huang
BAgr (Vet Med), MAgr (Vet Path), MSc, PhD, DACVP
Director of Diagnostics and Anatomic Pathologist
Prairie Diagnostic Services Inc.
Saskatoon, SK

Yanyun received his vet degree in South China Agricultural University (SCAU) in 2002, and Master of Agriculture degree (in Veterinary Pathology) in 2005 also in SCAU. He moved to Saskatoon in 2005 for further anatomic pathology training and obtained a MSc degree in 2008. Then Yanyun pursued a PhD program in the area of swine health and finished in 2013. Yanyun began to work in Prairie Diagnostic Services Inc. as an anatomic pathologist the same year. He is an ACVP boarded anatomic pathologist, and is also the Director of Diagnostics and interim CEO of PDS. Yanyun is currently conducting a research project using high throughput sequencing to detect viruses associated with bovine respiratory disease. Yanyun is married to his wonderful wife Vivi and has two boys, Eason (10) and Gideon (6).

Durda Slavic
DVM, MSc, PhD
Veterinary Bacteriologist
Laboratory Services Division.
Animal Health Laboratory
University of Guelph

Durda receive her DVM degree at University of Zagreb in Croatia. Two years after graduating, shed moved to Canada and started her MSc at Ontario Veterinary College in veterinary bacteriology working with *Actinobacillus suis*. *A. suis* was also the topic of her PhD work. In 2004, she was hired as a Veterinary Bacteriologist by AHL where she has been working since. Her areas of expertise are bacterial and fungal diseases and antimicrobial resistance.

More about our invited Speakers //

Plus sur nos conférenciers invités

Carl A. Gagnon
DMV, PhD
Directeur/Director – CRIPA
Laboratoire des maladies infectieuses virales vétérinaires
Laboratoire de diagnostic virologique vétérinaire et de diagnostic moléculaire
FMV, Université de Montréal

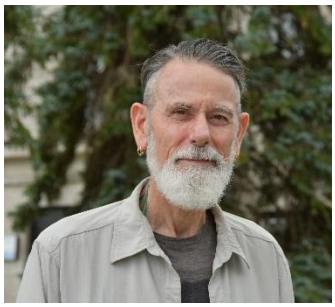
Dr. Carl A. Gagnon obtained a DVM in 1994 from the Faculté de médecine vétérinaire (FMV) of Université de Montréal. He then pursued graduate studies in virology and immunology and obtained a doctorate (PhD) in 2001 at the INRS Institut-Armand-Frappier under the supervision of Dr. Serge Dea, his thesis topic being the antigenic and genomic variations of porcine reproductive and respiratory syndrome virus (PRRSV). He was the director of the Center of Experimental Biology of INRS (research laboratory complying with GLP standards) from 2003 to 2004. Since 2004, he is a professor at the FMV, his research activities mainly targeting porcine virology. As scientific director of the Veterinary Virology Diagnostic Laboratory (VVDL) and the Molecular Diagnostics Laboratory (MDL) at FMV, he is daily involved in the diagnosis of animal infectious diseases (swine, avian, zoo and wildlife, etc.). Dr Gagnon has recently set up a new high throughput sequencing diagnostic laboratory in addition to be responsible of the Transmission electron microscopy service at FMV.

Oliver Lung
PhD, Research Scientist/Head, Genomics Unit Canadian Food Inspection Agency / Government of Canada
National Centre for Foreign Animal Disease

Dr. Oliver Lung obtained his Ph.D. degree from the Department of Molecular Biology and Genetics at Cornell University and received post-doctoral training on molecular virology at the Boyce Thompson Institute for Plant Research. In 2006, he joined the Canadian Food Inspection Agency (CFIA) Lethbridge Laboratory as a Research Scientist where his focused on developing automated user-friendly multiplex nucleic acid tests for detection and typing of viruses causing foot-and-mouth disease, classical swine fever, avian influenza and differential diseases. In Lethbridge, he also oversaw the Microarray facility and the OIE and Canadian reference laboratory activities for BVD and IBR and supported diagnostic testing for these and other bovine and equine viral diseases. In 2015, he moved to CFIA's high containment laboratory at the National Centre for Foreign Animal Disease in Winnipeg to establish a new Genomics Unit. In this role, he established a high throughput sequencing (HTS) facility in the containment level 3 laboratory to facilitate HTS of samples containing high consequence viruses processed in the CL3 and CL4 laboratories. His current work focuses on developing and applying HTS and bioinformatics analysis methodologies to support diagnostics, surveillance and research activities involving known and novel viruses.

More about our invited Speakers //

Plus sur nos conférenciers invités



John Morris Fairbrother
DVM, PhD,
Director of Reference Laboratory for *Escherichia coli*
Faculté de médecine vétérinaire,
Université de Montréal

Dr. Fairbrother graduated with a Bachelor of Veterinary Science in 1972 from the Faculty of Veterinary Science at the University of Sydney, Australia. He completed a doctorate in veterinary microbiology at the College of Veterinary Medicine at Cornell University in Ithaca, New York in 1981. He then joined the Faculté de médecine vétérinaire, Université de Montréal, Saint-Hyacinthe, Canada, building up a research program on the identification, pathogenic mechanisms and control, and more recently on antimicrobial resistance, of *E. coli* causing disease in animals. In 2003, he co-founded Prevtec microbia, a spin-off company of the Université de Montréal, specialized in the development and commercialization of preventive methods in animal production, including Coliprotec®, a live vaccine for swine production developed at the EcL. In 2006, Dr. Fairbrother was named as OIE Expert for *E. coli* and his laboratory was officially designated as the OIE Reference Laboratory for *Escherichia coli* by the World Organisation for Animal Health (OIE).

Marcelo Gottschalk
DVM, PhD,
Professeur/Professor
Faculté de médecine vétérinaire/Faculty of Veterinary Medicine
University of Montreal

-DVM (Buenos Aires, Argentina) and PhD (Montreal)
-Doctor Honoris causa (Ghent University, Belgium) for outstanding contribution to veterinary bacteriology
-Professor at the Department of Pathology and Microbiology, Faculty of Vet Med, University of Montreal
-Director of the international reference laboratory for *Streptococcus suis* and swine pleuropneumonia

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Younès Chorfi
DVM, MSc, PhD
Professeur agrégé (Nutrition et productions animales)
Département de Biomédecine Vétérinaire
Université de Montréal
Faculté de médecine vétérinaire

Dr Younes Chorfi received his doctorate in veterinary medicine in 1994 from the Agronomic and veterinary Institute, Rabat, Morocco. He went on to complete a master degree (1999) and a PhD (2005) at the Faculty of veterinary medicine University of Montreal where he is associate professor of “nutrition, feeding and animal production”.

Grant Maxie
DVM, PhD, Diplomate ACVP
Co-Executive Director,
Laboratory Services Division,
Director, Animal Health Laboratory,
University of Guelph

Grant Maxie was born and raised in southern Alberta. After graduating with his DVM from the Western College of Veterinary Medicine in Saskatoon, he completed an internship in small animal medicine and a PhD in clinical pathology at the Ontario Veterinary College. Following 3 years working in Kenya on a trypanosomiasis research project, he joined the faculty at the OVC as an anatomic pathologist, teaching cardiovascular and urinary pathology. After moving to the Veterinary Services Laboratory of OMAFRA in Guelph, he became board-certified in anatomic pathology by the American College of Veterinary Pathologists. He served as a veterinary pathologist and then as Guelph laboratory head for VLSB. He is currently the Director of the Animal Health Laboratory and co-Executive Director of the Laboratory Services Division at the University of Guelph.

Pursuing an interest in scientific editing, Grant was the editor-in-chief of the Canadian Veterinary Journal, has published extensively, and edited and co-authored chapters in the 5th and 6th editions of “Jubb, Kennedy and Palmer's Pathology of Domestic Animals". He is a past-president of the American Association of Veterinary Laboratory Diagnosticians (AAVLD), volunteered for many years as an assessor on the Accreditation Committee, and is the current editor-in-chief of the Journal of Veterinary Diagnostic Investigation.

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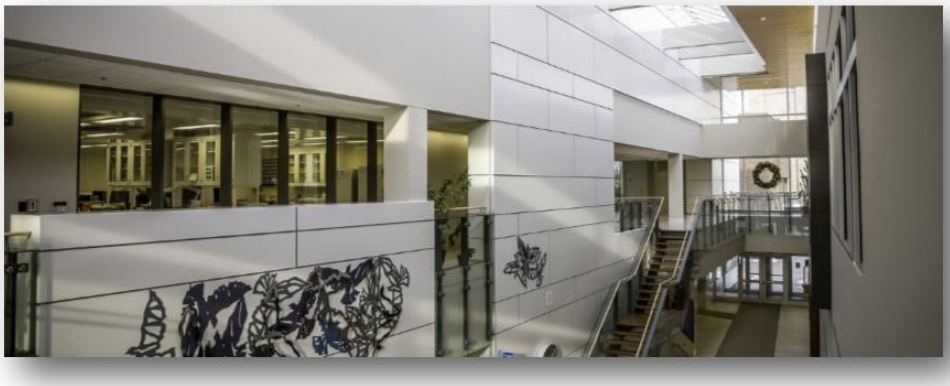
Since 2013, the Diagnostic Service is accredited by the *American Association of Veterinary Laboratory Diagnosticians* (AAVLD), ensuring quality for its customers.



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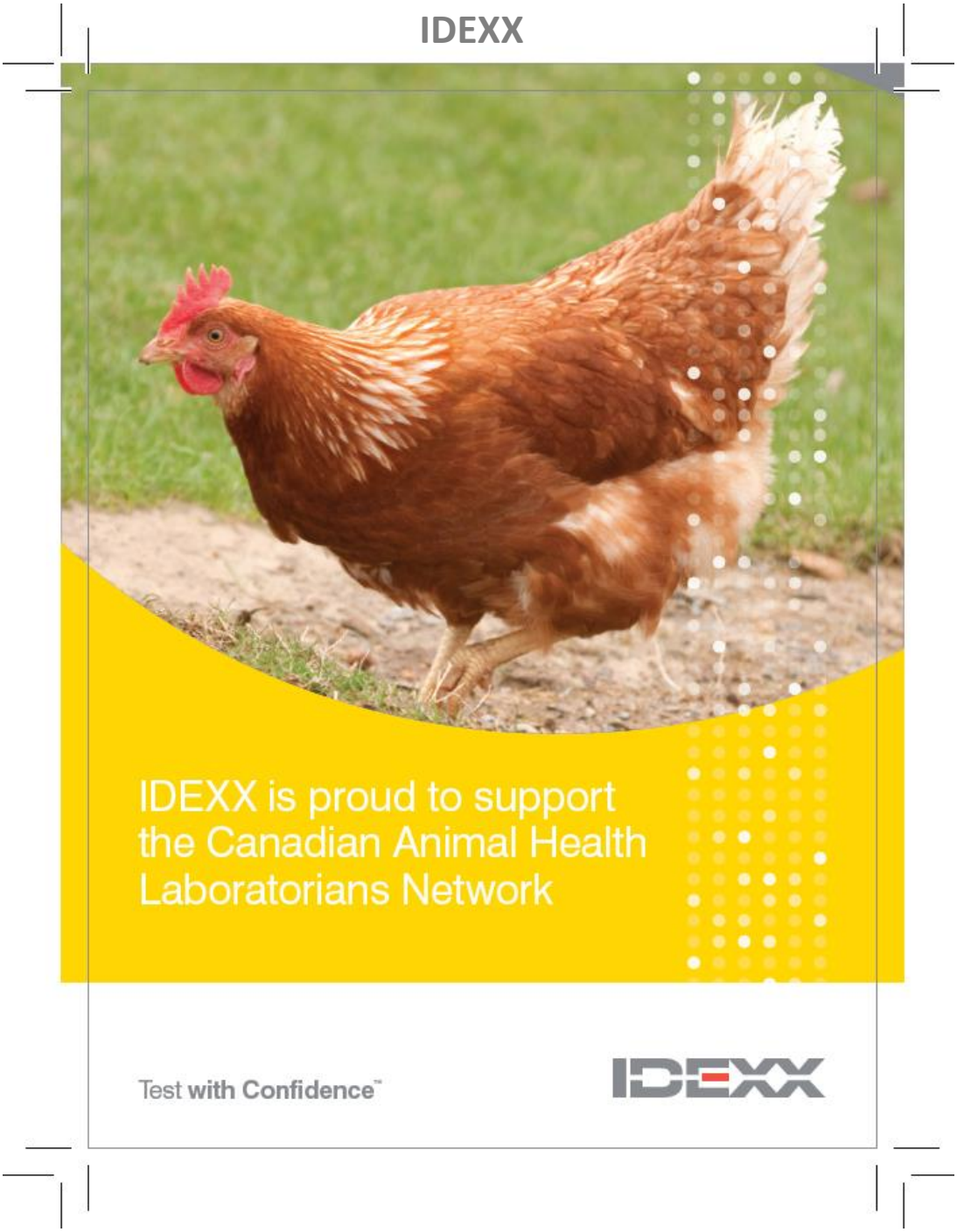

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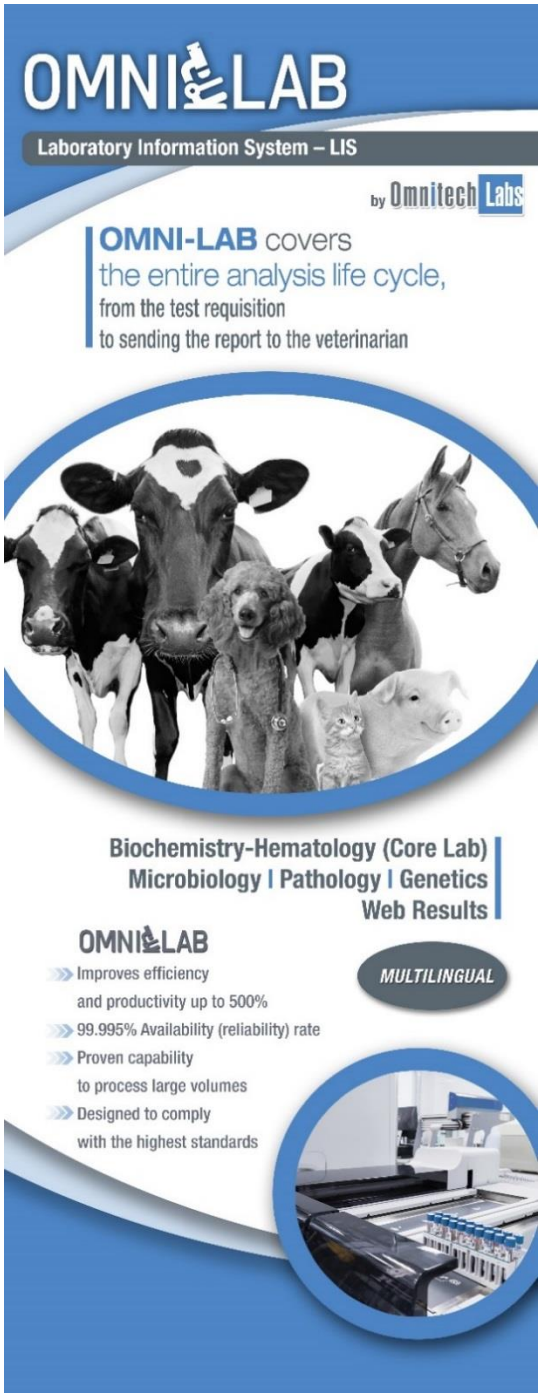

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[1] USDA, NAHMS - Dairy, 2008. [2] Ott SL, et al. Prev Vet Med, June 1999. [3] USDA, NAHMS - Beef, 1999.

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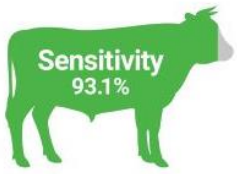

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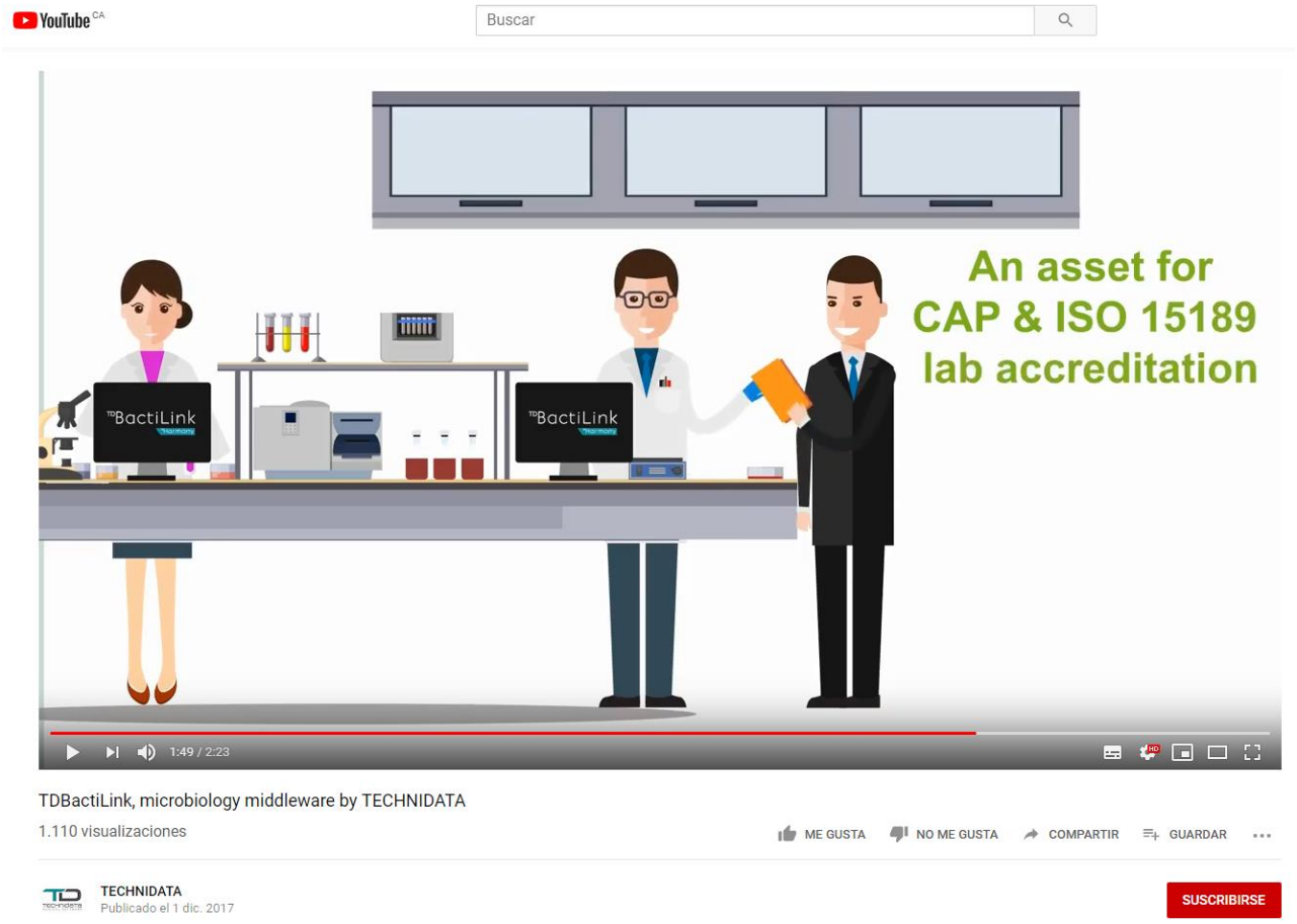

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Adapting high throughput sequencing in an animal health laboratory – where are we and where to go?

Yanyun Huang[1]

[1]Prairie Diagnostic Services Inc.

High throughput sequencing (HTS), also known as Next Generation Sequencing (NGS), is one of the developing frontiers in animal health laboratories. HTS as a diagnostic tool has many potentials, such as enabling non-targeted detection of bacteria and viruses in one sample, combining detection and genotyping of pathogens, providing pen-side testing with remote analyses in laboratories, and decreasing turnaround time among others.

However, HTS is not without significant challenges for animal health laboratories to adopt. Significant cost of initial investment, operational challenges to maintain steady test-load, and the need for further refinement of the technology are some of the challenges, just to name a few.

In this presentation, I will attempt to give an overview of HTS as a diagnostic tool. Starting with a survey of the current state of the application of HTS in some animal health laboratories in North America, and drawing on PDS's own still-evolving journey, I will propose some future directions for animal health laboratories to adapt HTS into diagnostic use.

In the my opinion, animal health laboratories can achieve steady HTS activities by conducting or contracting research projects, by partially switching some tests from traditional methods to HTS or by capitalizing on other opportunities best suited to the laboratory's own situation. Laboratories also need to further validate HTS, incorporate it into a robust quality system, and develop user-friendly, easy-to-understand, yet informative report formats.

HTS will likely become one of the frequently used diagnostic tools in the future. By working closely together, Canadian animal health laboratories can add this new tool into their toolbox and use it to safeguard North American animal industries and economic prosperity.

Pioneering a new technology in accredited veterinary diagnostic laboratory - things to look forward to, or not…

Durda Slavic[1], Davor Ojkic[1], Grant Maxie[1]

[1]Animal Health Laboratory, Laboratory Services Division, University of Guelph, Guelph, Ontario

Next generation sequencing (NGS) has proven to be a very valuable tool for research purposes, and many medical clinical laboratories have adopted this technology for routine detection of genetic disorders. In veterinary diagnostic laboratories, NGS has not yet been widely used for clinical purposes even though more and more laboratories have access to it. Bringing a new technology online presents many challenges for any laboratory and particularly for accredited veterinary diagnostic laboratories as rarely are there any guidelines on standardization and validation available.

Our laboratory acquired an Illumina MiSeq through the Veterinary Laboratory Investigation and Response Network (Vet-LIRN). This network includes multiple veterinary laboratories across the United States and Canada who are collecting bacterial isolates and compiling antimicrobial susceptibility data. Some of the isolates are sequenced and their sequences are uploaded into the GenomeTrakr database hosted at the National Center for Biotechnology Information (NCBI). As a part of the project, Vet-LIRN is ensuring harmonization of the methodology among the labs. This includes preparing and sharing standard operating procedures, providing genetically well characterized isolates for practice runs, and also providing proficiency testing samples. Our experiences with NGS method harmonization and validation through Vet-LIRN are in the early stages, but look very promising for both research and diagnostic purposes.

Day-to-day challenges in a high throughput sequencing diagnostic laboratory

Carl A. Gagnon[1], Chantale Provost[1]

[1]Centre de Recherche en Infectiologie Porcine et Avicole (CRIPA); and Molecular diagnostic laboratory (MDL), Faculté de médecine vétérinaire, Université de Montréal

Set up a new next-generation sequencing veterinary diagnostic laboratory (NGSVDL) and implement this technology on a routine base come with several challenges. In addition to sustaining the economic fitness of the laboratory budget, other challenges may arise. As an example, even if the training of highly qualify personal is important, you have to be able to keep the newly trained employees given the fact that other laboratories will try to recruit them!

To assure whole genome sequencing (WGS) of a specific virus from clinical samples, it is crucial to use DNA/RNA extraction protocols that will increase the targeted viral genome while reducing the genomic contaminants (mainly the infected host genome). The viral genome exists in several forms, while there are several extraction protocols that are more efficient with specific forms of viral genome. It is crucial to take advantage of each characteristic of the viral genome to increase the viral genome extraction efficiency from a clinical sample. While some next-generation sequencing laboratories may focus on improving genome extraction protocols from clinical samples, others are purifying viruses contains in clinical samples and so, are reducing significantly the genomic contaminants. An old forgotten method used to purify viruses from clinical samples, that is easy to use with a short running time, will be presented. Moreover, some types of clinical samples, such as serum and saliva, contain significantly lower amount of genomic contaminants. If possible, those types of clinical samples should be favored if WGS of viruses is requested by the laboratory customers.

The knowledge of NGSVDL end-users can be highly advance or unfortunately, rudimentary. The end-users lack of knowledge may lead the ethical problems and other issues that need to be address. As an example, an end-user may add foreign virus DNA in a sample as a DNA control. Unfortunately, if to many foreign virus DNA is poured into a sample, it can hide the real wanted virus target. The training of end-users can be a key step toward overcoming those issues and will ensure, for sure, their satisfaction toward NGSVDL services.

High throughput sequencing at Canada's Only Containment Level 3 HTS Facility: The first 3 years

Oliver Lung[1]

[1]Canadian Food Inspection Agency, National Centre for Foreign Animal Disease

Novel, emerging/re-emerging and exotic infectious diseases represent a growing challenge for both public and animal health authorities worldwide. The Canadian Food Inspection Agency's National Centre for Foreign Animal Disease (NCFAD) located in Winnipeg, Manitoba is a laboratory mandated with surveillance, diagnostic testing, diagnostic test development and scientific advice regarding high consequence infectious diseases related to animals. The laboratory has biosafety containment levels (CL) 2, 3 and 4 laboratories for diagnostic testing, diagnostic test development and research. This presentation will include an overview of the CL3 HTS sequencing facility at NCFAD and ongoing activities. Examples of the methodologies used for sequencing of a broad range of priority viruses, as well as unknown, or unexpected pathogens in support of diagnostic, surveillance and research activities will also be presented.

Development of a whole genome sequencing approach for identification and antimicrobial resistance of pathogenic *Escherichia coli* in the veterinary diagnostic laboratory

John Morris Fairbrother[1], Ghyslaine Vanier[1], Maud de Lagarde[1], Gabriel Desmarais[1]

[1]OIE Reference Laboratory for *Escherichia coli*, Service de Diagnostic, Université de Montréal, Faculté de médecine vétérinaire, St-Hyacinthe, Canada

Multidrug resistant pathogenic *E. coli* cause substantial economic losses in the intensive pig and poultry production industries. Current diagnostic techniques are time consuming, costly, incomplete, and lack resolution, being unable to identify and track all pathogenic isolates and clones. The overall aim of this project is to set up and validate genomic tools for rapid detection and identification of pathogenic and antimicrobial resistant *E. coli* in the veterinary diagnostic laboratory.

We have set up and carry out whole genome sequencing (WGS) on the Illumina MiSeq platform (Illumina, San Diego, CA) using a Nextera XT library. Reads are assembled using SPAdes genome assembler via Illumina BaseSpace Sequence Hub. In silico analysis is performed for virulence genes, antimicrobial resistance genes, multilocus sequence types (MLST), serotypes, and plasmid replicon types using the Bacterial Analysis Pipeline Tool (GoSeqIt and Center for Genomic Epidemiology (http://www.genomicepidemiology.org/)). Assemblies are screened for additional virulence genes using Galaxy tool or BLAST. This approach is now available as a diagnostic test in our laboratory.

We are currently using this approach, together with core genome MLST and single nucleotide polymorphism (SNP) phylogenetic analysis, to examine pathogenic *E coli* isolates from clinical cases in pigs and chickens belonging to different pathovirotypes and commensal *E. coli* isolates from the intestinal tract of normal healthy animals of the same species. This will permit us to more precisely define the profile of a pathogenic isolate and to identify markers for the development of more rapid and less costly screening tests. In addition, WGS has permitted the identification of the emergence of a new clone of fluoroquinolone resistant ETEC:F4 associated with more severe cases of diarrhea in preweaning and postweaning pigs.

More complete and comprehensive typing of *E. coli* isolates will improve diagnostic feedback to veterinarians and will permit a more accurate monitoring of trends and identification of risk factors to help veterinarians and industry in developing effective control strategies while diminishing the use of antimicrobials.

Genetic diversity preliminary results of swine influenza virus in Quebec, Canada, in 2018

Chantale Provost[1], Carl A. Gagnon[1]

[1]Université de Montréal

Swine influenza virus (SIV) is one of the leading causes of respiratory disease in pigs. Moreover, SIV can cause sporadic zoonotic infections posing a potential risk to human and pig health. It is therefore important to follow its evolution and study its characteristics. The objective of this study is to understand the evolutionary dynamics of SIV currently circulating in Quebec's swineherds. To achieve this goal, 18 submitted samples, following respiratory illness emergence in swine herds that were subsequently found SIV RT-qPCR positive, were selected and sequenced by high throughput sequencing (HTS) using Illumina technology (MiSeq). Moreover, samples from four different genotypes (H3N2, H1N1, H1N2, and H3N1) were selected for HTS. Ten H3N2, 6 H1N1, one H1N2, and one H3N1 SIV strains were fully or partially sequenced. Genetic constellation shows that of the 10 H3N2 sequenced, 6 different reassortment profiles were identified, and of the 7 H1N1 sequenced, 4 different reassortment profiles were identified. Phylogenetic trees illustrated that all (n=7) H1 gene classified within the SwH1β cluster, all (n=7) N1 gene classified within pH1N1 cluster, all (n=10) H3 gene classified within cluster IV, all (n=11) N2 gene into porcine-North-American cluster, all (n=21) M gene into pH1N1 cluster. The other genome fragments have also been classified. Moreover, 4 co-infections were observed in clinical samples, one clinical sample had H1N1 and H3N2 genome fragments present, one sample had two different M, NP, and NEP/NS genes, and two samples had two different M genes. Antigenic sites analysis show that H1 gene product differ between 0 and 4 within all 6 antigenic sites and that H3 gene product differ between 2 and 10 within their 5 antigenic sites. All N1 genes possessed the mutation N70S, which is known to induce Zanamivir resistance. All N2 were exempt of known mutations to induce NA inhibitor resistance. All strains sequenced also have the highly frequency molecular marker S31N, which is associated with resistance to M2 blocker, Amantadine. Whole genome sequences study of SIV will allow a better understanding of their evolution, diversity, and characteristics. Thus, eventually this will help diminish sporadic zoonotic infections, preventing a potential risk to human and pig health.

A customizable high-throughput genotyping technology that permits fast, easy, and inexpensive alteration of marker content

Jason Wall[1], Claudio Carrasco[1], Krishna Reddy Gujjula[1], Hakten Suren[1], Prasad Siddavatam[1], Christopher C. Adams[1]

[1]Thermo Fisher Scientific, 2130 Woodward Street, Austin TX 78744

Attractive and valuable high-throughput genotyping solutions for parentage and breeding applications require the ability to simultaneously interrogate hundreds to thousands of genetic loci both easily and economically. One disadvantage of many high-throughput genotyping technologies is the lengthy lead times and considerable cost associated with changing the genomic marker content (targeted loci) in any particular assay. The Applied Biosystems™ AgriSeq™ targeted genotyping-by-sequencing (GBS) solution for plant and animal genotyping does not suffer from this problem because the technology relies on a pool of PCR oligonucleotides that can be quickly, easily and inexpensively changed to accommodate always improving knowledge of genomic function. If and when the need arises to alter the content of a marker panel all that is required is the design and synthesis of additional PCR primers which are then simply spiked into existing assay pools. In addition, AgriSeq genotyping panels can be ordered in plate format in which primer pairs for marker-containing amplicons are individually aliquoted enabling the user to drop unneeded amplicons or re-formulate primer pools (panels) in any combination desired. Furthermore, individual panels targeting specific species can mixed together, creating a multi-species panel, while still enabling species-specific genotyping. For example, a mixture of three mid-density panels for multiple species not only allowed for accurate species-specific genotyping, but also enabled the accurate assignment of species to unknown gDNA samples being tested. This unparalleled flexibility in a highly multiplexed genotyping platform provides users unlimited avenues for customizing their genotyping workflows

For Research Use Only. Not for use in diagnostic procedures.

Illumina: Advances of Microarray and NGS in Animal Breeding and Health

Kahlil Lawless[1]

[1]Agrigenomics Segment Manager, Americas, Illumina Inc

Over the last 20 years Illumina has developed the most powerful DNA sequencing and microarray technologies in the world. Researchers and Animal Testing Experts use these technologies to profile and manipulate animal genetics, and in animal pathogen research and outbreak management. In addition, these technologies offer powerful methods for understanding animal pedigrees and health information, such as identifying animals who are carriers of pathogenic genetic mutations. Ultimately, just as NGS has become a powerful FDA approved tool for human diagnostics, we may see similar test development in the veterinary diagnostics field.

In this talk we will seek to explain how Illumina microarray and next generation sequencing technologies work, how they continue to evolve and improve while costs come down, and explore examples where scientists are using this technology to conduct animal research, apply results to breeding strategies and farm management, or in the offering of consumer tests. We will also profile how genetic information is being used to catalogue species biodiversity, and how this is being increasingly leveraged in the management of natural habitats or industrial impacts.

Using molecular tools to identify previously undetectable causes of ovine abortion - *Helicobacter trogontum*

Maria Spinato[1], Margaret Stalker[1], Durda Slavic[1]

[1]University of Guelph

A producer with a flock of 250 Bluefaced Leicester and Scottish Blackface sheep reported 3 abortions of near-term fetuses in a group of 52 breeding ewes. This group of crossbred ewes had been recently purchased and relocated from Saskatchewan. Two additional abortions had occurred previously; however, fetuses were at an earlier stage of gestation, and diagnostic testing was not pursued. Ewes appeared to be healthy, in good body condition and had been recently vaccinated and dewormed. The ration consisted of corn silage, oats and free choice hay. Samples of fresh placenta obtained from two abortions were submitted to the Animal Health Laboratory, University of Guelph for diagnostic testing. Cotyledons in one placenta were noted to be mildly swollen. Samples of placenta were submitted for culture for common bacterial pathogens including *Campylobacte*r, PCR testing for *Coxiella* and *Chlamydia*, and histopathologic examination. No significant bacterial pathogens were isolated in culture, and PCR tests for *Coxiella* and *Chlamydia*were negative. Histologic features in both placentas were similar: there was moderately advanced autolysis and trophoblastic epithelium was sloughed. Extensive infiltrates of neutrophils were present within both cotyledonary and intercotyledonary chorioallantois. In one placenta, several allantoic arterioles were undergoing leukocytoclastic vasculitis, associated with occasional thrombosis. Based upon the histologic findings of marked suppurative placentitis, further testing for infectious causes of ovine abortion was initiated. PCR testing for *Toxoplasma* and *Leptospira* was negative, and immunohistochemical staining for BVD/Border disease virus was also negative.

Reports in the literature of sporadic cases of ovine abortion due to *Flexispira rappini* in New Zealand and the United States initiated PCR testing of saved frozen portions of placenta using genus-specific *Helicobacter* primers. The obtained PCR product was sequenced and sequence results (100% coverage and 99.4% identity) revealed the presence of *Helicobacter trogontum*, previously known as *Helicobacter* (*Flexispira*) *rappini*.

This is the first identification by the Animal Health Laboratory of *Helicobacter trogontum* as a cause of placentitis and abortion in sheep. Whether this organism is a sporadic, previously undetected or emerging cause of infectious ovine abortion in Ontario remains to be elucidated. Much remains unknown regarding the source of the organism, the method of transmission and its pathogenicity. Molecular tools have now provided us with the means of answering some of these questions.

Review of necropsy cases in Québec

Isabelle St-Pierre[1]

[1]Laboratoire de santé animale Québec, MAPAQ.

Presentation of Quebec Animal Health Laboratories activities in 2018 including number of submissions by species, number of analysis by sectors, number of submissions for necropsy, main diagnosis in production animals.

Interesting cases in Québec

Isabelle St-Pierre[1]

[1]Laboratoire de santé animale Québec, MAPAQ.

Presentation of interesting cases submitted in Quebec Animal Health Laboratories in recent years.

PISAQ: Abortion in small ruminants

Magaly B-Pépin[1], Dominique Fournier[1], Geneviève Côté[2], Anne Leboeuf[2]

[1]Laboratoire de santé animale, Québec, MAPAQ ; [2]Direction générale des laboratoires et de la santé animale, Sous-ministériat à la santé animale et à l'inspection des aliments, MAPAQ.

We explain the functioning and main results obtained so far within the PISAQ (Programme intégré de santé animale du Québec) campaign investigating infectious causes of abortion in small ruminants in Quebec.

One of the main goal is to improve productor's and veterinarian's knowledge about the infectious agents most commonly implicated in small ruminant's abortions. Ultimately, the productors, with the assistance of their field veterinarians, may put sanitary mesures in place to help reduce the reproductive losses due to those agents in their herd and, most importantly, may help reduce the transmission from animals to human. This program will also gives us some epidemiological datas about the incidence of infectious diseases affecting the small ruminants herd located in our territory that could influence our future preventive actions or research priority.

So far, *Chlamydia sp*, *Toxoplasma gondii* and *Coxiella burnetti* have been implicated most frequently (respectively for 18%, 15% and 10%) in abortions cases submitted in our lab since the beginning of the program in October 2017.

Postmortem Laboratory FAD Emergency Exercise - First experience

Véronique Boyer[1], Sonia Chénier[2], Julie-Hélène Fairbrother[2]

[1]Service de diagnostic, Faculté de médecine vétérinaire, Université de Montréal. [2]Laboratoire de santé animale, Ministère de l'Agriculture, des pêcheries et de l'alimentation du Québec (MAPAQ) et Service de diagnostic, Faculté de médecine vétérinaire, Université de Montréal.

The diagnostic laboratory in St-Hyacinthe (CDEVQ) is situated in a new building (2013) and includes a very active postmortem room and a foreign animal disease (FAD) diagnostic containment level section. The laboratory is part of the Canadian Animal Health Surveillance Network (CAHSN) and the analysts in the FAD diagnostic containment level section are certified by the National Center for Foreign Animal Disease (NCFAD) annually to fulfill the tests for diseases such as avian influenza (AI). Standard operating procedures (SOPs) provided by the NCFAD are made available for the FAD this laboratory and these are tested with annual panels.

On the other hand, SOPs used in the postmortem room are written internally and they had never been challenged with FAD emergency exercises. Rapidly identifying a FAD can significantly reduce the economic damages that are associated with the infection, but management of the investigation is also crucial.

On May 9th 2019, we organized our first emergency exercise to test and validate the SOPs regarding management of a reportable FAD in the postmortem laboratory. Another purpose was to evaluate how every employee reacts to such a suspicion and test joint coordination and communication among CDEVQ and the different external contributors (CFIA, MAPAQ) involved in an emergency situation. The simulation staged a suspected avian influenza outbreak in a poultry flock and we had the privilege to observe many steps of the exercise. Our main observations and challenges will be shared and presented. An assessment from an external evaluator and the after-action plans will also be provided.

Diagnosis of *Streptococcus suis* in an affected farm: harder than expected

Marcelo Gottschalk[1]

[1]Groupe de Recherche sur les Maladies Infectieuses en Production Animale and Diagnostic Service.

According to the Canadian Swine Health Information Network, *Streptococcus suis* (*S. suis*) related diseases are the most common infectious problem reported on Canadian swine farms. Outbreaks of *S. suis* disease result in decreased performance and increased mortality which have a significant economic impact on swine production in our country. Clinical features associated with *S. suis* infection in pigs are meningitis, arthritis, endocarditis, polyserositis and septicemia with sudden death. There are 35 serotypes that have been described, although some of them have been now re-classified as non-*S. suis*. Although a few virulent serotypes are recovered from diseased animals in Europe (mostly serotypes 2 and 9), the situation is much complicated in Canada and USA, where a relatively large number of serotypes are routinely isolated from diseased pigs, with no clear predominance of a given serotype.

Recent data from Quebec, Canada and USA indicate that serotypes 1, 1/2, 2, 3, 5 and 7 (depending the region) may be the most important, but these will count all together for less than 60% of the isolates recovered from diseased pigs. Multilocus sequence typing (MLST) may give some indication of the virulence of such isolates: those included in the clonal complex 1 (mostly sequence type or ST 1) are considered highly virulent. In USA and Canada (at least in Quebec), between 10 and 20% of recovered isolates (serotypes 1, 2 and 14) belong to this ST. Although ST28 serotype 2 strains have been traditionally considered as low virulent in experimental infection in animal models, most field strains of serotype 1/2 (predominant serotype in USA and Canada) as well as those of serotype 2 (all recovered from ill animals) belong to this ST. This may indicate that results obtained in experimental infections do not always reflect the reality of the field.

Diagnosis of *S. suis* as a primary pathogen is indeed difficult, mostly in North America. A combination of several samples taken from different animals, serotyping, MLST (if appropriate) and PCR testing for some virulence markers (for specific serotypes) must be used before performing a final diagnosis. Although the use of autogenous vaccines needs still to be scientifically proven, if the isolate chosen is not the one causing most cases of the disease in a herd, no protection will be obviously obtained. Indeed, the diagnosis may significantly influence the effectiveness of such vaccines.

Measuring deoxynivalenol (DON) in animals

Corinne Généreux[1], Younès Chorfi[2]

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Animal feeds are frequently contaminated with various mycotoxins produced by the secondary metabolism of diverse fungal contaminants. Among these mycotoxins, deoxynivalenol (DON), also known as vomitoxin, is the most frequent mycotoxin occurring in animal feed. Distribution of DON in the feed is heterogeneous and the sampling is a large source of variability associated to reliable results of DON concentration. Thus, mycotoxin measurement in feeds has limitation, because it depends on the type and size of the sampling performed, and on the sensitivity of the tests used. The objective of this study was to develop analytical method to measure DON and its metabolite depoxy-DON (DOM-1) in animal serum; and ultimately establish a cause-effect relationship between the presence of DON and DOM-1 in animal serum and the symptoms attributed to a possible DON contamination /intoxication. Serum DON and DOM-1 were measured by reverse phase HPLC method using immunoaffinity column. In pigs, in dairy cows and dairy heifers, the results showed that serum concentrations of DON and DOM-1 were highly correlated to the concentration of DON in the diets (R2= 74%, 68% and 74%). Measurement of serum DON and DOM-1 is therefore a reliable method to evaluate DON contamination/intoxication in animals.

Selenium levels in Quebec horses

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Soils in Eastern Canada are notably devoid of selenium (Se) and animals consuming locally grown feeds are at risk of becoming deficient unless supplemented. Quebec horses are mostly fed locally grown forage and cereal grain which typically contain Se at a concentration ≤ 0.05 ppm on dry matter (DM) basis, however Se requirement for horses has been estimated at 0.1 to 0.3 ppm DM. A method of direct measurement of serum Se was validated using reverse phase high performance liquid chromatography (HPLC). Data of Se analyses submitted at the Faculty of veterinary medicine from 2014 to 2018 show that the majority of submitted samples are under the adequate level of 1.7 µmol/l with 77%, 83%, 79.4%, 79% and 70% of horses respectively. Among these horses, 19% (2014 and 2015), 23% (2016), 20% (2017) and 14% (2018) were highly deficient with serum concentration below 0.67 µmol/l. These results show that Supplementation practices commonly employed in Quebec are inadequate to ensure selenium sufficiency in horses.

Development and validation of an indirect ELISA for the serological diagnosis of African swine fever

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African swine fever (ASF) is an economically devastating viral disease of pigs caused by African swine fever virus (ASFV), a large enveloped DNA virus. The currently available commercial ASF ELISA kits are developed and licenced in Europe. Given the recent and rapid spread of ASF, there is a critical need for ASF diagnostic tests to be developed in North America. Three ASFV antigenic surface proteins, p30, p54 and p72, were expressed in recombinant baculovirus system and were evaluated in an indirect enzyme-linked immunosorbent assay (iELISA) for the detection of anti-ASFV antibodies using known anti-ASFV antibody negative serum and serum collected from pigs experimentally infected with different ASFV strains. A receiver operating characteristic curve (ROC) analysis of the data demonstrated that the p54 has the highest sensitivity (99.5%) and specificity (95.5%) of the three recombinant proteins, and was able to detect anti-ASFV antibodies in experimentally infected animals as early as 8 days post-infection (DPI). The p54 iELISA is a promising tool for the serological diagnosis of ASF. This novel iELISA is currently in the process of full validation in collaboration with a commercial partner.

Respiratory viruses identified in western Canadian beef cattle by high throughput sequencing and their association with bovine respiratory disease

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Bovine respiratory disease (BRD) causes significant economic losses in western Canada despite viral vaccination and massive antimicrobial treatment. The pathogenesis involves interactions between bacteria, viruses, environment and management factors. Primary viral infection can greatly increase susceptibility of beef cattle to bacterial infection, and is thus a vital part of BRD pathogenesis. The objective of this study was to use metagenomic sequencing to characterize the respiratory viromes of paired nasal swabs and tracheal washes from western Canadian feedlot cattle, with or without BRD. A total of 116 cattle (116 nasal swabs and 116 tracheal washes) were analyzed. Based on results generated from MiSeq, Illumina, the presence of influenza D virus (IDV), bovine rhinitis A virus (BRAV), bovine rhinitis B virus (BRBV), bovine coronavirus (BCV) and bovine respiratory syncytial virus (BRSV) was associated with BRD. Agreement between identification of viruses in nasal swabs and tracheal washes was generally weak, indicating that sampling location may affect detection of infection. Subsequently, Nanopore sequencing was applied for seven selected samples and the results showed 100% agreement of virus detection with those by MiSeq. This study reported several viruses for the first time in Canada and provides a basis for further studies investigating candidate viruses important to the prevention of BRD.

Generation of Capripoxvirus vaccines to differentiate vaccinated animals from unvaccinated animals and validation of a competitive enzyme linked immunosorbent assay to test vaccine efficiency

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Capripoxvirus (CapV) represents a genus of the poxviridae family which includes: sheep pox virus, goat pox virus, and lumpy skin disease affecting sheep, goats, and cattle respectively. CapVs are considered World Organization of Animal Health (OIE) notifiable because of their classification as agri-terrorism agents. These viruses have caused and continue to cause irreparable damage to animals and directly affect economies of countless developing and developed countries.

Currently the main method of control is through vaccination using attenuated live field isolates. Due to the immune response generated by the animals to the vaccine, it is not possible to serologically test whether or not an animal has been vaccinated. Differentiating infected from vaccinated individual vaccines are a promising means to control and monitor the spread of CapVs.

This project involves the use of the Romanian strain of sheep pox virus vaccine and the Neethling lumpy skin disease virus vaccine strain by Onderstepoort Biological Products to generate these vaccines. The process involves knocking-out a non-essential gene in the vaccines which will result in a unique antibody response from vaccinated animals lacking antibodies to the protein of interest. The knock-out process involves the use of homologous recombination and more recently CRISPR and molecular diagnostic tools including full genome sequencing to validate generation of mutant virus. This is done in parallel with the validation of a competitive enzyme linked immunosorbent assay (cELISA) developed in house, which will detect the difference in antibody response.

So far, a modified lumpy skin disease virus has been generated and will be followed by characterization of the newly generated mutant, animal trials and further molecular and serological tests to prove vaccine efficiency. We aim to show that these vaccines not only provide full protection against challenge with a pathogenic capripoxvirus strain, but also that they will allow for a differentiation of vaccinated and unvaccinated animals using the serological assay validated. The development of the modified vaccines along with use of the cELISA test and the possible development of a molecular test will allow for better control and monitoring of disease as well as a simple accessible diagnostic method to differentiate vaccinated from infected animals lessening the burden that countries affected by CapVs face in terms of trade restrictions.

Whole genome sequencing of porcine reproductive and respiratory syndrome virus (PRRSV) from clinical samples

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Porcine reproductive and respiratory syndrome virus (PRRSV) is a major economic concern worldwide, costing over 150 million dollars yearly to the Canadian swine industry alone. There are currently large data sets available about the ORF5 gene of the virus (which represent only 4% of the entire viral genome), with thousands of sequences available from around the globe, but little data is currently available on the full-length genome of PRRSV. We hypothesized that whole genome sequencing (WGS) of PRRSV genome would provide a better insight into the pathogenicity of different strains and would allow for a better epidemiological monitoring compared to ORF5 sequencing. Therefore, PRRSV positive serum, saliva and lung tissue samples submitted to the Molecular diagnostic laboratory (MDL) at the "Faculté de médecine vétérinaire (FMV)" were used to sequence the entire viral genome of PRRSV. Those samples, originating mostly from Quebec, were submitted to the diagnostic laboratory for routine surveillance or the diagnosis of PRRSV infection. The RT-qPCR Ct values of the samples sequenced were varying between 10 and 32. The viral genomes were isolated, and libraries were prepared using Nextera XT technology. They were sequenced on an Illumina Miseq sequencer. 91 full length PRRSV genome were obtained from 111 samples.

First, three important deletions in the ORF1a, totalling 523 nucleotides, were found in most non-vaccine-like strains from Quebec. The importance of these deletions remains to be determined.

Also, comparisons of maximum likelihood phylogeny trees for both the ORF5 and the full genome showed that using the whole genome offers a better resolution and higher degree of confidence in tree topology.

Interestingly, the full-length genome of two different co-infecting PRRSV strains were found in four different samples, suggesting a 4.60% prevalence of PRRSV co-infection within clinical samples. The two strains in each of these samples shared an identity at the nucleotide level between 81.83 and 92.36 %. Moreover, 6 PRRSV strains were also found to cluster differently (more then 2 nodes away) based on using only the ORF5 or the whole genome.

Thus, WGS of PRRSV enables a better classification and/or interpretation of results in 11.19% of clinical samples compared to ORF5 sequencing, as well as open-up interesting research avenues.

Detection of Foot-and-Mouth Disease Virus in Swine Meat Juice

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Foot-and-Mouth Disease Virus (FMDV) is a picornavirus that infects various cloven-hoofed livestock causing foot-and-mouth disease (FMD) that manifests as fever, reduced appetite, and blisters. FMD plagues agricultural industries worldwide as a result of greatly reduced production and trade embargoes and poses a constant threat to countries that have been able to obtain the FMD-free designation by the World Organization for Animal Health (OIE). Conventional methods of FMD detection and diagnosis rely on samples such as blood collected while an animal is still alive. In cases where an animal is discovered after death or when animal meat is imported illegally, typical sample matrices are no longer viable. Meat juice is a potential sample substrate that can be collected in these unique circumstances and used for detection of FMDV. Animal experiments were conducted at the National Centre for Foreign Animal Diseases (NCFAD) on infected pigs to determine whether the virus can be detected in meat juice. Meat juice was collect from biceps femoris and the diaphragm and tested for FMDV by three different Real-time Reverse Transcriptase Polymerase Chain Reactions (RRT-PCRs) and Lateral Flow Immunoassay. Preliminary findings show that FMDV RNA can be detected by all three RRT-PCRs as early as one day after infection, and as late as twenty one days after infection. Similarly, viremia was detected one day after infection, but was cleared in all remaining pigs by day 12. FMDV antigen was also detected in meat juice by lateral flow strip test. These initial results show that meat juice is a viable sample type for FMDV detection.

Multiplex real-time PCR assay for three psittacine viruses

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Aves polyomavirus-1, psittacine beak and feather disease virus, and psittacid herpesvirus-1, are DNA viruses that can cause substantial morbidity and mortality in psittacine birds and more efficient diagnostic tests are needed to better manage these diseases in captive populations.

The purpose of this project was to develop and validate a multiplex real-time PCR (rtPCR) test to be offered through the Animal Health Laboratory at the University of Guelph.

Using publically available nucleotide sequences, primers and probes were designed to amplify highly conserved regions of each virus. Diagnostic validation was performed on archived formalin-fixed, paraffin-embedded samples from psittacine birds diagnosed at postmortem through the University of Guelph with naturally occurring infections of these three viruses.

Diagnostic cases used for validation totaled 85 birds. Virus-positive birds showed histopathologic evidence of infection (i.e., characteristic viral inclusion bodies in tissues), and virus-negative birds showed no signs of viral infection, and were selected from cases with postmortem final diagnoses unrelated to the pathogens of interest. The assay had high analytical sensitivity, detecting less than six copies of viral DNA per reaction, and 100% specificity, showing no cross-reactivity with 59 other veterinary agents. The multiplex rtPCR assay confirmed 98% of histopathology-positive cases, and also identified many subclinical infections that were not detected by histological examination, including coinfections. Results of histopathology and rtPCR showed high agreement overall and were confirmed by kappa statistics. Cycle threshold values were significantly lower in birds with viral inclusion bodies compared to rtPCR-positive birds without inclusion bodies.

Overall, this new assay has been demonstrated to be effective at confirming these DNA viral infections in psittacine birds diagnosed by histopathology, as well as to identify silent or latent infections, and provides an effective tool for diagnosis of psittacine disease.

Evolution of veterinary lab testing – view from the trenches

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I've seen marked changes over my 50 years of experience in veterinary labs, as an undergrad student at WCVM, graduate student in OVC, clinical pathologist in Kenya, anatomic pathologist in Guelph, lab director, and laboratory quality assessor. Major highlights include consolidation of provincial labs, growth of private labs, major gains in efficiency though automation, changes in technology, and improved lab design. Rudimentary laboratory equipment has been replaced by more sophisticated automated equipment – faster, cheaper, smaller! Computerized laboratory information management systems have grown and improved – hardcopy and fax have been displaced by email and Web access to results. Barcoding and Excel spreadsheets have facilitated high-throughput testing. Immunohistochemistry has been a boon to anatomic pathologists. Virus isolation has been displaced by PCR. FA testing has shrunk. MALDI-TOF MSs have taken over bacteriology labs. Autosamplers allow overnight test runs. Lab accreditation systems have matured – validated tests are mandatory, and continued competence must be documented. The value-added activity of integrating surveillance data nation-wide is becoming a reality. What does the future hold – what will be the role of provincial laboratories in remote sensing, point-of-care testing, machine learning, metagenomics, big data?

Cutting Edge Technology: direct field testing using a portable instrument without nucleic acid extraction

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Worldwide, Central Reference Laboratory systems are being overwhelmed by the volume of African Swine Fever (ASF) samples being submitted. The surge volume is causing substancial delays in the reporting which confounds real-time decision making for control efforts. Here we demonstrate results of cutting edge technological developments that facilitate on site testing.
For FMD, two independent real-time RT-PCR assays targeting different regions (5'UTR and 3D) of the FMDV genome were used. A robust sample preparation method for serum, esophageal-pharyngeal fluid and epithelial suspensions was developed to negate the need for RNA extraction prior to rRT-PCR. In a field study in Africa, results from a common set of samples tested by lyophilized reagents on the T-COR8™ platform and the gold-standard laboratory-based rRT-PCR were compared.
ASF - In Uganda, Blood and/or tissue samples were collected from domestic pigs during outbreak investigations in five districts of Uganda. A dried-down ASFV PCR kit with internal control (IC) (Tetracore Inc., Rockville, Maryland) was used on a portable real-time PCR thermocycler T-COR 8™ (Tetracore Inc.), was deployed in a austere lab setting in affected villages. As a reference, the OIE recommended UPL assay (Fernández-Pinero et al 2013) was performed on a Stratagene Mx3000P at NADDEC.
FMD - The final rRT-PCR protocol and associated lyophilized reagents were field evaluated in three endemic settings (Kenya, Tanzania and Ethiopia), consistently detecting both clinical and subclinical FMD infections. Results of 145 samples tested in three test sites combined showed a 100% correlation between results between lab-based and field-based results.
ASF - Pigs from two of the five suspected outbreak sites investigated were positive for ASFV using the ASF kit on the T-COR 8™ and these results matched those of the reference method in the lab at NADDEC with 100% correlation.
Current delays in reporting from Centralized Reference Laboratories confound real-time decision making for animal control and disease continement efforts. Technical advances demonstrated are: dried assays stored at ambient temperaturs; new chemistries that allow direct testing; and mobile, fieldable PCR instruments. A timely and accurate molecular diagnosis under field conditions would support outbreak investigation and enable authorities to take appropriate actions to control and prevent transboundary animal diseases including ASF. Decentralized, on-site testing methods are undergoing validation efforts with OIE Reference Laboritories to enable the adoption of the technology by National Animal Disease Control authorities.

Building a new AMR surveillance system in Canada: AMRNet

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Antimicrobial resistance (AMR) is a growing concern at a global level. The rise of pathogens resistant to all classes of antimicrobials from both human and animal sources, coupled with the lack of new effective antimicrobial agents, has resulted in infections that do not respond to antimicrobial therapy.

The Public Health Agency of Canada has identified this issue as a priority and has established a framework for moving forward. The key areas of focus include surveillance, antimicrobial stewardship, infection control, and research. Surveillance provides important data that can identify specific risks associated with acquisition of AMR infections and provides important baseline data that can be used to identify emerging threats and measure the effects of interventions. There are key gaps in our surveillance of AMR pathogens. Of major importance is the lack of community data concerning AMR, surveillance in small hospitals, nursing and long-term care facilities, and northern regions.

Antimicrobial susceptibility testing for both human and animal isolates is being conducted in hundreds of public and private laboratories across Canada on a daily basis for individual patient/animal care and to ensure the appropriate use of antimicrobials (stewardship). The data is stored in electronic format in laboratory information management systems (LIMS) across Canada, but in general is not used beyond direct patient care with the exception of generating hospital antibiograms.

In this presentation I will be describing a new surveillance program called AMRNet that will electronically capture the existing AMR data in human and possibly veterinary laboratory information systems, in addition to existing antimicrobial use data, that would be electronically transferred to a centralized database under the existing Canadian Network for Public Health Intelligence (CNPHI). CNPHI has been used to securely capture and store information for many surveillance programs including FluWatch, the Canadian Nosocomial Infection Surveillance Program (CNISP), and the PulseNet Program. Once in CNPHI the data would be converted to a standardized format for analysis as laboratories use different LIMS systems and data fields. The data will be used to identify emerging and/or extensively drug resistant AMR pathogens, capture community level AMR data, assist in Canada's international AMR reporting obligations to the WHO, and assist in antimicrobial stewardship programs.

Canadian Animal Health Technology and Intelligence Network (CAHTIN)

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The Canadian Animal Health Technology and Information Network (CAHTIN) will vastly improve Canada's ability to rapidly detect, analyze, and respond to new and emerging diseases (such as foot-and-mouth disease, African swine fever, and zoonotic influenza) that can spread very quickly and cause devastating impacts on animal health, human well-being, and the Canadian economy. By building on the existing, but very fragmented (and often informal) animal health networks, CAHTIN will create the much needed national framework linking Federal, Provincial, and Territorial government agencies, stakeholders along the entire food animal chain, and academic expertise. A not-for-profit corporation leveraging significant member experience in research and product development will lead the network.

CAHTIN will drive seamless integration of laboratory diagnostics, field surveillance, and intelligence analysis across all animal species, production systems, processing networks, and distribution value chains. Early detection surveillance systems facilitated by a distributed diagnostic network utilizing novel point-of-care and lab-based technologies will be the key to identifying and containing the threat before it spreads, managing a disease outbreak, reducing antimicrobial resistance, demonstration sound animal welfare and production practices, and ultimately, certifying disease freedom– a critical pre-requisite for global market access and the national economy sustainability. This is especially important for the pork and beef export sectors.

We are at the edge of a new era. By combining the expedience of nanopore metagenomic diagnostic analysis (in labs across the country) with extensive epidemiological intelligence, CAHTIN will create a powerful utility for tracking food-borne pathogens, and for promoting and monitoring prudent antibiotic use in animal agriculture, benefiting both animal and human health. By linking these diagnostic surveillance and intelligence analysis systems, CAHTIN can supply the vaccine experts at the VIDO-Intervac vaccine research group with the most up to date genomic information regarding pathogen population and emergence in the field. This creates an unprecedented capacity for rapid new vaccine development, enhancing both antibiotic use reduction and general infection control.

The CAHTIN model will bring cohesion to Canada's animal health system by building a sustainable national partnership among publicly funded diagnostic laboratories, surveillance networks, research centres, agri-businesses, academia, governments and biologics developers. Through collaboration, gaps and overlaps in the system will be addressed, all stakeholders will benefit directly and the competitiveness of Canadian animal agriculture as a whole will be increased.

Labgenvet.ca, a bilingual web-based resource for genetic diseases in domestic animals

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Labgenvet.ca is a web-based resource designed to facilitate DNA genotyping and provide information regarding genetic disease of domesticated animals. **Labgenvet.ca** was developed at the Faculty of Veterinary Medicine (FVM), University of Montreal, and provides its services in both English and French. Genotyping is provided by the **Laboratory of Veterinary Genetics** (**Labgenvet**), which is a level 1 diagnostic lab housed at the FVM. **Labgenvet** receives samples taken from domestic animals for DNA genotyping for known genetic diseases and traits. Genetic tests are available for the canine, feline, bovine, equine and avian species, and samples are received from Quebec, from Canada and internationally. Samples are typically buccal swabs or blood, but can also be hair and feathers. Genomic DNA is extracted via standard methods, the site of the allele of interest is amplified by PCR, and amplicons are sequenced via Sanger methods. Processing of samples typically takes between 4 to 5 days, but can be as rapid as two days. About three quarters of samples received by **Labgenvet** are sent by animal breeders and come from healthy animals that may or may not be carriers for a given genetic disease. In this case, genotyping is useful for making management decisions with the goal of improving the overall genetic health of the breed in question. About one quarter of samples received by **Labgenvet** are sent by veterinary clinics and often come from animals that are showing particular clinical signs. In this case genotyping is of medical diagnostic value. The percentage of samples received by **Labgenvet** coming from veterinary clinics is increasing as Canadian veterinarians become more educated regarding genetic diseases in domestic animals and more aware that a Canadian diagnostic lab is now available. In addition to diagnostics, the **Labgenvet.ca** web site has a major educational mandate and maintains a searchable data bank for genetic diseases in domestic animals. For example, in dogs, more than 150 disease and trait mutations are documented and over 240 specific breeds are represented. For cats, 27 disease and trait mutations are documented and over 40 specific breeds are represented. For each disease, a link provides additional genetic and clinical information and further links provide access to the scientific literature. Genetic diseases can be searched for by disease name, by breed and by medical system. These functions are provided as an educational resource for veterinarians, for veterinary students, for animal breeders and for the general public.

Optimizing the immunocytochemistry technique

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With a regular Giemsa stain, cells from different lineage can look alike or share similar features, making their accurate identification difficult or impossible. However, a more precise diagnosis can impact the patient's prognosis. All cells express many markers on their surface, within their cytoplasm or nucleus. Some are specific or can be suggestive of a cell type and several are routinely used as immunohistochemistry (IHC) diagnostic tools to further characterize a lesion. Identical markers can theoretically be applied on cytology specimens for the same purpose. Yet, there are some constraints with cytological samples such as the small/restricted number of available slides, the fact that most slides are often already stained, and the variable cellularity from slide to slide.

The objective of this study was to validate and standardize the immunocytochemistry (ICC) technique for selected markers: CD3, CD20, Vimentin (Vim), Cytokeratin (CK) and Von Willebrand factor (Vwf). Air-dried Giemsa-stained cytology samples were selected based on their likely expression of the chosen marker. It was decided to develop ICC on previously stained samples in order to mimic a realistic diagnostic setting with a limited number of slides. Megakaryocyte-rich samples, such as spleen and bone marrow smears, were chosen for the expression of Vwf. Carcinoma and sarcoma cases were selected in order to test CK and Vim respectively. Lymphomas were chosen for the expression of CD20 and CD3. When evaluating the ICC staining, three criteria were targeted: adequate conservation of the cellular features, including nuclear shape (in order to properly identify the population of interest), optimal marker expression (pattern and intensity as theoretically expected on the cells of interest) and minimal background noise/non--specific staining. Most steps of the manual IHC technique were slightly modified in order to optimize the ICC results. Namely, the following parameters were adjusted: fixation, antigen retrieval, buffers, antibody concentrations and exposure times, as well as hematoxylin counterstain.

We identified that the antigen retrieval step and the antibody concentrations were the two main steps to be adjusted based on the manual validated IHC.

Rapid detection of bovine respiratory bacterial pathogens and associated antimicrobial resistance profiles by whole-metagenome sequencing of clinical samples

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Bovine respiratory disease (BRD) is one of the most costly condition of cattle responsible for 65 to 80% morbidity and 45 to 75% mortality in some feedlots. Treatment and prevention of BRD lead to extensive use of antimicrobials in the cattle industry. Unfortunately, antimicrobial resistance (AMR) has been identified as an important public health threat worldwide due to the emergence of multidrug resistant bacteria. The world organization for animal health promotes prudent use of antimicrobials and recommends that these drugs be prescribed only when the etiological agent has been identified and the antimicrobial susceptibility test (AST) performed. However, the current laboratory diagnosis and AST for BRD treatment and metaphylaxis guidance is not a common practice due to long laboratory turnaround time (TAT; 3 to 5 days) from sample submission to availability of results. The purpose of this study was to assess the feasibility for a rapid detection of BRD pathogens and their AMR profiles directly from diagnostic samples by whole-metagenome sequencing (WMGS) using Nanopore platform. Thirty samples including deep nasal swabs and lungs from feedlot calves with or without respiratory infection were cultured for the most common BRD pathogens *Mannheimia haemolytica* (Mh), *Pasteurella multocida* (Pm) and *Histophilus somni* (Hs). AST of isolates was determined by the broth microdilution method to the common BRD antimicrobials used in the cattle industry: ampicillin, ceftiofur, danofloxacin, enrofloxacin, florfenicol, gamithromycin, penicillin, spectinomycin, tetracycline, tildipirosin, tilmicosin, trimetroprin-sulfamethoxasole and tulathromycin. Furthermore, these samples were tested for the presence of BRD pathogens and associated AMR gene profiles by WMGS. We recovered 33.3% of Mh and 10.0% of each of Pm and Hs from bacteriology cultures. In contrast, we detected more BRD pathogens by WMGS (Mh-43.3%; Pm-13.3% and Hs-20.0%) as compared to culture. In addition, other pathogens such as *Bibersteinia trehalosi* (6.6%) and *Mycoplasma bovis* (23.3%) not isolated from culture, were detected by WMGS. The AST phenotype of isolates was comparable to the genotype profile obtained by WMGS. Our data indicate that WMGS can provide laboratory diagnostic information on BRD pathogen identification and AMR genotype similar or better to that obtained from routine culture and AST. Interestingly, this diagnostic information can be obtained faster using WMGS (5 hours) as compared to culture (3 to 5 days). Therefore, WMGS may be considered as a tool that improves diagnostic laboratory TAT and subsequently, may guide clinicians towards better treatment options and decision on prudent use of antimicrobials.

Diagnosis of canine urinary tract infection; laboratory versus in-clinic test accuracy to guide antimicrobial selection

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Antimicrobial resistance (AMR) is a global public and animal health crisis recognized by the World Health Organization (WHO). Currently, it is estimated that AMR bacterial infection is responsible for 700,000 human deaths annually and if no action is taken, this number could reach 10 million by 2050. Commitment to fight AMR received the highest level of political endorsement at the United Nations General Assembly. The resulting WHO Global Action Plan and Pan-Canadian AMR Framework challenge animal health scientists and diagnosticians to provide support for enhanced antimicrobial stewardship in veterinary medicine. The National stewardship initiatives include changes to Canadian regulations since December 2018 that requires prescriptions for the sale of all medically important antimicrobial drugs (AMD) for use in animals. Three key objectives have been identified to help inform prudent antimicrobial usage (AMU) in animal healthcare and animal agriculture: Streamlining and revising classical laboratory methods to expedite turnaround time and diagnostic information handling; Exploring novel technologies for benchtop and point-of-care applications to provide accurate and rapid diagnostic test results to veterinarians; Strengthening professional communication and continuing education. The purpose of this study was to evaluate the use of AMD in veterinary clinics for urinary tract infection (UTI) in dogs. We have collected 108 urine samples from suspected cases of UTI, based on 'in-clinic' sediment analysis, from veterinary clinics across Saskatchewan. Afterwards, the dogs were treated with antimicrobials as per clinic protocols. The samples were submitted to the Prairie Diagnostic Services (PDS) laboratory the day of collection for urinalysis (UA), bacterial culture and antimicrobial susceptibility test (C&S). Among these samples, UTI was confirmed in 63 (58.3%) by culture; however 45 (41.7%) were negative. There was 95.4% agreement between PDS laboratory UA and C&S results. The most pathogens isolated included *Escherichia coli* (49.2%), *Proteus mirabilis* (22.2%) and *Staphylococcus pseudintermedius* (12.7%). The majority of these pathogens were susceptible to the AMD recommended as empiric first choice for uncomplicated UTI, which some clinics did not necessarily follow. Our results indicate that AMD are inaccurately administered in approximately 42% of dogs suspected of UTI. The main reason given by clinicians for this practice was time delay (2 to 3 days) between sample collection and availability of laboratory results. These observations call for diagnostic laboratories support of the antimicrobial stewardship program by exploring novel technologies to provide accurate and rapid diagnostic information that may guide clinicians towards better treatment options and decision on prudent use of antimicrobials.

Distribution of *Streptococcus suis* (from 2015 to 2018), *Actinobacillus pleuropneumoniae* and *Haemophilus parasuis* (from 2017 to April 2019) isolated from diseased pigs in Quebec

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Streptococcus suis is an important pathogen of swine and serotype determination remains a valuable tool for veterinary practitioners and/or diagnosticians to understand the epidemiology of the infection and to evaluate the need for serotype-specific autogenous vaccines in a herd. We present here the distribution of serotypes among 583 isolates of *S. suis* recovered from diseased pigs from Québec in the last four years. Serotypes 1/2 and 2 were the most commonly detected. Other frequently detected serotypes were (in decreased order) serotypes 7, 3, 4, 5, 9 and 8. Previous reports indicated around 20% of untypable isolates; however, we identified only 8.2% of untypable *S. suis* among the tested isolates. The possible explanation is that we test now untypable isolates to confirm if they belong to the *S. suis* species: indeed, 7.2% of isolates tested were not "true" *S. suis* and were not considered as untypable. Characterization of virulence markers of serotype 2 isolates is presently underway.

Porcine pleuropneumonia caused by *Actinobacillus pleuropneumoniae* (App) is a contagious disease reported to cause economic losses worldwide (4). Serotyping is still of major interest for App since different serotypes have different virulence potential, depending on the geographical origin. A total of 109 isolates, recovered from diseased animals were serotyped. Serotypes 7 (48) and 5 (30%) were by far the serotypes most frequently identified. Surprisingly, serotype 12 (usually considered as a low virulent serotype) followed with almost 10% of the isolates. Other serotypes found were serotype 8, 2, 6 and 15.

Haemophilus parasuis, the etiological agent of Glässer's disease, is one of the most important bacterial pathogens of swine. It usually causes polyserositis, but infection may also result in arthritis, meningitis, and septicemia. Serotyping of this pathogen is important for decisions on vaccination strategy to prevent future outbreaks as bacterins which are usually used induce mainly serotype-specific immunity. A total of 69 isolates of *H. parasuis* recovered from diseased pigs were received from January 2017 to April 2019. Serotypes 5/12 (which cannot be differentiated by PCR), 4 and 7 were the most commonly found. These serotypes have also been described as highly prevalent in other countries and are considered high virulent. Only 10% of isolates were non-typable.

Our laboratory is specialized on the serotyping of these pathogens, and we also receive many samples from other laboratories in the USA. It is important to keep these data updated to better understand the epidemiology of these infections.

An introduction to the Canadian Animal Health Surveillance Network (CAHSN) laboratory support team and the implementation of the African Swine Fever (ASF) proficiency testing program

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The Canadian Animal Health Surveillance Network (CAHSN) is a network of laboratories across Canada, developed to build diagnostic capability for the detection of emerging foreign animal disease (FAD) threats, provide rapid response and generate surge capacity. The diagnosis of FAD is the mandate and responsibility of the CFIA. However, with the ability of provincial and university laboratories to collaborate in disease surveillance, the early warning system created greatly improves the country's ability to respond to an outbreak.

The National Centre for Foreign Animal Diseases (NCFAD) provides the expertise on the diagnostic FAD tests performed by the CAHSN laboratories, including notifiable avian influenza, Newcastle disease, foot-and-mouth disease, classical swine fever, bluetongue and African swine fever. The NCFAD diagnostic sections provide ongoing assay performance and improvements to ensure the most up to date test methods are transferred to the CAHSN laboratories.

The laboratory support team, working out of the NCFAD, provides the proficiency testing program developed to train and certify the CAHSN analysts performing the FAD testing for the network. The proficiency testing program has been designed based on, and strives to follow, the regulations outline in the ISO/IEC 17043 international standards. The team produces and quality tests technical certification panels, assay controls and reagents used in proficiency and diagnostic testing for three ELISA tests as well as five real-time PCR tests distributed to the network.

In response to the current threat of African swine fever (ASF), testing for ASF was added to the scope of assays performed by the CAHSN laboratories. Working in collaboration with the NCFAD diagnostic group, test method documentation was developed, testing material for the panels and assay controls was designed and generated, and a training plan was put into place to implement the new testing program for the network laboratories. With the need to have CAHSN laboratories prepared for emerging FAD threats, the laboratory support team was able to execute the plan and has successfully prepared the network for ASF testing.

Molecular characterization of complete genome of newly emerging avian reovirus variants and novel strains in Quebec, Canada, 2016-2017

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Avian reovirus (ARV) are the etiological agent of viral arthritis syndrome, also known as tenosynovitis disease causing significant economic impact on the poultry industry in Canada and USA. An upsurge in positive cases of ARV was observed in Quebec between 2016 and 2017. Thus, in this study, the ARV viral genome of positive clinical cases from that specific period were characterized to elucidate the cause of this increase. One hundred forty-nine ARV PCR positive tendons and feces clinical samples were randomly selected for isolation on chicken embryonic kidney (CEK) cells. From 66 ARV isolated strains which were inducing a cytopathic effect (CPE) in CEK infected cells, 43 strains with high CPE were subsequently selected for high throughput sequencing. They were sequenced with a MiSeq instrument, to recover their full-length genome. Of the 43 strains sequenced, 18 full-length genomes were obtained, 9 had some fragments recovered completely but other only partially, 4 had only the S1 gene fully sequenced, and 12 had all 10 genome fragments just partially sequenced. ARV strain genomes were characterized and their relationship with reference strains was examined. Based on standard classification found in the literature for ARV, genotyping clusters of partial δC gene (828 nucleotides), which are cluster I-V, were determined. Fifteen strains were classified within cluster I, 7 strains within cluster II, one strain within cluster IV, and 8 strains within cluster V. Phylogenetic trees of the 10 full length coding sequences and identity matrices were also done. However, to our knowledge no well establish cluster has been previously described for the other genome fragments of ARV, but some groups referring to old strains of ARV have been proposed. Our results seem to demonstrate that for S2, S3, S4, L1, L2, L3, M1, M2, and M3 genome fragments we have observed 4, 3, 2, 3, 3, 1, 4, 4, and 3 different groups, respectively. The molecular characterization of complete genome of newly emerging ARV strains remains an avenue that needs to be further investigated, as the literature is very limited at the moment.

Caractérisation d'*Escherichia coli* entéropathogènes (EPEC) isolées de porcs du Québec par séquençage de nouvelle génération

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Les *Escherichia coli* entéropathogènes (EPEC) peuvent causer la diarrhée post-sevrage chez le porc. Ces dernières possèdent le locus d'effacement des entérocytes (LEE), qui code pour les gènes de virulence nécessaires à la production de lésions caractéristiques d'attachement-effacement (A/E). Certains de ces microorganismes sont également résistants à plusieurs familles d'antibiotiques et ont la capacité d'acquérir cette résistance par échange horizontal. L'utilisation d'antibiotiques chez les porcelets malades pourrait contribuer à cette résistance. L'obtention du génome complet par séquençage de nouvelle génération Illumina permet la caractérisation en profondeur de ces souches. L'observation de dix souches séquencées permet d'obtenir un aperçu général de ce à quoi la population des souches EPEC porcines du Québec pourrait ressembler et ainsi ouvre la voie au développement d'un nouvel outil de diagnostic pour la détection des EPEC porcines.

L'ensemble des 10 souches sont des EPEC atypiques. Basée sur l'ensemble des données obtenues à partir du génome complet, 2 groupes de souches de caractéristiques similaires sont prédominantes. Ainsi 3 des 10 souches EPEC sont O45 :H11 de phylogroupe B1, ST29, alors que 2 des 10 souches séquencées sont O123 :H11 de phylogroupe B1, ST29. Basé sur leur profil de gènes de virulence, 9 des 10 souches ont le locus d'effacement des entérocytes complet, alors qu'une des souches possède locus LEE de façon partielle. À l'exception de deux souches, l'ensemble des EPEC analysées sont des ST29. De plus, selon leur profil de résistance aux antibiotiques, la moitié des souches EPEC sont multi-résistantes.

La caractérisation génétique par séquençage de nouvelle génération Illumina de 10 souches porcines EPEC a permis d'obtenir un aperçu et une meilleure connaissance des caractéristiques associées à ce pathotype. Éventuellement, ces connaissances permettront d'identifier des marqueurs biologiques spécifiques à ce type de souches, dans le but de développer des tests diagnostiques rapides et peu couteux.

Quebec Infectious bronchitis virus (IBV) strains genomic variability from 2011 to 2019 based on the S1 viral gene sequencing

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IBV S1 gene sequencing has been done in our laboratory for the poultry industry and veterinary practitioners since 2011. Until now, our databank contains 308 IBV S1 sequences, of which 271 were obtained between 2017-2019. This increase of 95% of sequencing request could be explain by the emergence of the IBV DMV strain in Quebec in 2017. This new strain represent 54% of the sequences contains in our databank, and it is the most frequent strain type found in IBV positive cases since 2017. Other strains identified are shared between: Mass41 and Conn46 vaccinal stains (25%); 4/91 strains (12.5%); Qu16 strains (5.5%); and CA1737 and GA2013 american variants (3%). Moreover, The DMV S1 sequences seem to be the less variable (≥ 96.4%) compared to the S1 gene of Mass41 (≥ 94.5%) and 4/91 (≥ 91.1 %) strains. Also, Quebec DMV strains are different from DMV/1639/11 USA reference strain (homology of 94.7% to 97.3%). Based on epidemiological factors (years of sample submission, veterinarian submitting the samples, city origin of the sample), homology between Quebec DMV strains seems to be unpredictable. Thus, since DMV strains caused economical and clinical impacts in the last few years, the Molecular diagnostic laboratory (MDL) of Faculté de médecine vétérinaire (FMV) developed a new RT-qPCR assay for the specific detection of IBV DMV strain. This DMV RT-qPCR assay was very sensitive and allows the monitoring of this specific strain in all kinds of samples including environmental samples, creating a rapid diagnostic and monitoring tool for the poultry industry.

Ureaplasma diversum, a bovine pathogen identified for the first time in the respiratory tract of swine in Canada

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Ureaplasma diversum (*U. diversum*) is an important bovine pathogen associated with cattle diseases. In bulls, it may cause seminal vesiculitis, balanopostitis and alterations in spermatozoids, whereas in cows, it may cause pneumonia, placentitis, fetal alveolitis, abortion and birth of weak calves. During the validation of a new qPCR diagnosis assay for the identification of *U. diversum* in bovine clinical samples, 133 samples of different type and different species were used to determine the sensitivity and specificity of this assay. Interestingly, two porcine lung samples were found positives for *U. diversum*. These samples came from two unrelated clinical cases, a boar and a piglet, which were submitted to our diagnostic laboratory in 2015 and 2016, respectively. At necropsy, the animals presented a pulmonary parenchyma congestion. There was edema in the alveoli and the interlobular septa and infiltration of macrophages and neutrophils into the alveoli. In general, the lesions indicated the presence of broncho-interstitial pneumonia with bronchial-associated lymphoid tissue (BALT) hyperplasia. *U. diversum* has been poorly documented in pigs worldwide and it had not been previously reported in pigs in Canada. The presence of *U. diversum* in the swine samples was confirmed by an external reference laboratory, using a general PCR for the detection of *Ureaplasma sp.* and a qPCR specific to *U. diversum*. These results were confirmed through sequencing the PCR amplicons obtained with two different sets of primers. The PCR amplicon nucleotide (nt) sequences had 98% similarity with *Ureaplasma sp.* and 99% similarity with *U. diversum*. To our knowledge, this is the first report of *U. diversum* in the respiratory tract of swine in Canada. High throughput sequencing analyses are currently done to obtain the complete genome of *U. diversum* swine strains to determine their phylogenetic relationship compared to those identified in bovine.

Emergence récente d'un clone ETEC : F4 non-susceptible à l'enrofloxacine dans la population de E. coli pathogènes chez le porc au Québec.

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Depuis 2013, nous avons observé une augmentation du nombre d'isolats ETEC:F4 provenant de porcelets malades (maternité et post-sevrage) soumis au laboratoire Ecl, dont environ 2/3 sont non-susceptibles à l'enrofloxacine. Nous avons démontré l'émergence d'un potentiel groupe clonal, en 2015, basé sur le profil PFGE des isolats. Notre objectif est d'étudier la clonalité de ces isolats grâce au séquençage du génome complet.

Cinquante-sept isolats ETEC:F4 ont été séquencé avec la technologie Illumina. L'analyse phylogénétique a été réalisée avec le logiciel Ridom SeqSphere+, basée sur le multi locus sequence typing du génome de base (cgMLST). Les bases de données du Centre d'Epidémiologie Génomique (CGE, http://www.genomicepidemiology.org/) et de l'Institut de Biologie Pathogène (Beijing, Chine, http://www.mgc.ac.cn/) ont été utilisées pour déterminer la présence de gènes de virulence, de résistance, de mutations ponctuelles et de plasmides.

Nous avons distingué 4 groupes différents. Le groupe d'intérêt principal est composé de 23 isolats, du sérotype O149, du Séquence Type 100, multirésistants et possédant des mutations des gènes *parC* S80I et *gyrA* S83L, associées à la non-susceptibilité à l'enrofloxacine. Les isolats appartenant aux trois autres clusters ne possèdent pas ces deux mutations.

Nous avons confirmé la présence d'un groupe clonal d'isolats ETEC:F4 non-susceptible à l'enrofloxacine qui circulent chez les porcs au Québec depuis 2013. Ce groupe clonal présente plusieurs caractéristiques des clones multirésistant à haut-risque. Ainsi, il est capital de surveiller son évolution pour évaluer les dangers qu'il pourrait représenter pour la santé du cheptel porcin et la santé publique.

Five years of monitoring pathogenic Escherichia coli virulence and antimicrobial resistance profiles in pigs in Québec

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Escherichia coli is an important cause of disease in swine causing neonatal and post-weaning diarrhea and oedema disease responsible for considerable economic losses due to mortality and morbidity. As OIE Reference Laboratory for *Escherichia coli*, one of our mandates is to monitor trends in virulence profiles and antimicrobial resistance (AMR) of pathogenic *E. coli*.

Between 2014 and 2018, more than 1377 *E. coli* isolates from over 2300 clinical cases in pigs submitted to the Diagnostic Service of the Faculté de médecine vétérinaire (FMV) of the Université de Montréal were characterized. These isolates were examined by multiplex PCR for the detection of the presence of genes coding for the 12 virulence factors most frequently found in pathogenic *E. coli*in pigs including toxins, and fimbrial adhesins F4 (K88) and F18 (F107). Prevalence of pathogenic *E. coli*, belonging to the enterotoxigenic *E. coli* (ETEC), enteropathogenic *E. coli* (EPEC), Shiga toxin-producing *E. coli* (STEC), and extraintestinal pathogenic *E. coli* (ExPEC) pathotypes was also reported. In addition, antimicrobial resistance (AMR) was determined by the standard disk diffusion assay on Mueller-Hinton agar at the Laboratoire de Santé Animale (LSA-MAPAQ) or Diagnostic Service of the FMV. Finally, demographic and clinical data were gathered.

Even though ETEC:F4 remained the most prevalent pathovirotype in Québec, different types of ETEC:F4 were observed throughout this 5-year period. Hence, an emerging LT:STb:STa:F4 virotype and the previously predominant LT:STb:F4 virotype were responsible for most of the ETEC:F4 diarrhea cases from 2014 to 2016. Subsequently, in 2017 and 2018, a third virotype, STa:STb:F4 has been more frequently observed. On the other hand, prevalence of other pathogenic *E. coli* such as STEC:F18, causing oedema disease, decreased. Finally, pathotypes such as ETEC:F18 and ETEC:STEC:F18, which were previously only infrequently isolated, are now more regularly found.

Overall, more than 90% of the ETEC:F4 isolates were multi-drug resistant, LT:STb:STa:F4 and LT:STb:F4 isolates being mostly non-susceptible to enrofloxacin, whereas STa:STb:F4 isolates were enfloflaxacin susceptible and demonstrated a distinct AMR pattern (neomycin and trimethoprim-sulfisoxazole resistance). Other emerging pathovirotypes, such as ETEC:F18, were also multi-resistant (around 80%) but susceptible to enrofloxacin. Globally, these results underline the need for continuous surveillance of pathogenic *E. coli* for virulence profiles and AMR in the pig.

Evaluating an automated clustering approach of porcine reproductive and respiratory syndrome virus (PRRSV) field strains for ongoing surveillance

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Porcine reproductive and respiratory syndrome virus (PRRSV) has a major economic impact on the swine industry. Since the important genetic diversity needs to be considered for disease management, ongoing surveillance of circulating endemic strains is beneficial. The objective of this project was to classify Quebec PRRSV ORF5 sequences in genetic clusters and evaluate stability of clustering results over a three-year period using an in-house automated clustering system.

Phylogeny based on Maximum likelihood (ML) was first inferred on 3661 sequences collected in 1998-2013 (Run 1). Then, sequences collected between January 2014 and September 2016 were sequentially added into 11 consecutive runs, each one covering a three-month period. For each run, detection of clusters, which were defined as groups of ≥15 sequences having a ≥70% rapid bootstrap support (RBS) value, was automated in Python. Cluster stability was described for each cluster and run based on the number of sequences, RBS value, maximum pairwise distance and agreement in sequence assignment to a specific cluster.

First and last run identified 29 and 33 clusters, respectively. In the last run (n=4958), 77% of the sequences were classified by the system. Most clusters were stable through time, with sequences attributed to one cluster in Run 1 staying in the same cluster for the 11 remaining runs. Some initial groups were further subdivided into subgroups with time, which is important for monitoring since one specific wild-type cluster increased from 0% to 45% between 2007 and 2016. This automated classification system will be integrated into ongoing surveillance activities.

An efficacy trial with dietary chlortetracycline against respiratory pasteurellosis in farmed rabbits

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Canadian rabbit farming currently lacks approved antibiotics to fight bacterial diseases. Pasteurellosis is an important disease causing 40% mortality and higher morbidity rates in grower rabbits if left unattended. Based on *P. multocida* in vitro sensitivity and oral pharmacokinetics of tetracycline in rabbits, we hypothesized that dietary chlortetracycline (CTC) would be effective and safe at controlling pasteurellosis in grower rabbits.

We performed a randomized blinded study in two commercial rabbit farms (140 rabbits/farm), where weaner rabbits were sorted in cages of 1 male and 1 female (35 rabbits/sex/treatment/farm). Treatments with medicated feed containing 880 mg/kg CTC hydrochloride versus unmedicated feed were started when the disease prevalence reached 10%. Treatments lasted for 7 days, with 14 days of follow-up. Clinical signs and feed intake were monitored daily. Dead and moribund rabbits were necropsied for histopathological and microbiological analysis. Statistical analysis was performed on blinded data.

Groups showed significant differences in mortality rate (53% vs. 22% in treated animals; P=0.0006): survival plots showed that untreated rabbits died during the first 10 days, while most treated rabbits died during the first 3 days (P<0.0025). Growth rate increased with weekly feed intake (P<0.0001), antibiotic treatment (P=0.017) and in animals considered healthy at treatment onset (P=0.0024). We isolated *P. multocida* from lesions in the nasal cavity, tympanic bullae, and lungs. No adverse effect of CTC was noted during the treatment period.

These results support the use of chlortetracycline as a safe and effective therapeutic option for Canadian rabbit farmers and veterinarians to fight respiratory pasteurellosis.

Optimization of the efficiency of viral infection for two porcine nidoviruses (PRRSV and PEDV) of veterinary importance

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Our research project is focused on emerging swine pathogens of unprecedented economic significance, such as the porcine reproductive and respiratory syndrome virus (PRRSV) and porcine epidemic diarrhea virus (PEDV). We hypothesized that the composition of PRRSV and PEDV virions and proteomic profiles of virally infected cells should reflect the complex interplay between the virus and host factors, which shape the course of infection. Host-virus interactions are highly dynamic, thus, a certain optimization is needed in order to investigate the spatio-temporal regulation of viral infection.

To address our hypothesis, we proposed: synchronize and increase virus entry into the cells and study the proteomic patterns of infected cells in a time-resolved mode, and produce and purify PRRSV and PEDV using the simian cell culture that is routinely used for PRRSV/PEDV production and porcine cells. Optimization of the efficiency of viral infection is a high importance for a variety of research applications, diagnostics and vaccine production. The calculation of titers of virus stocks and the estimation of viral load in clinical samples for many viruses often involve cytopathic effect (CPE) quantification in plaque-forming units (PFU) or similar approaches. Interestingly, it was shown that virus infectivity can be enhanced several-fold by chemical and physical methods. For example, positively charged molecules (polycations) and spinoculation are routinely used for lentiviral gene transfer, and it was shown that both approaches increased the infectivity of retroviruses. Thus, polycations can be used for rapid detection of viruses and cost-effective manufacturing of vaccines.

Here, we demonstrated that polycations greatly enhanced the efficiency of nidovirus entry and infection. Thus, polycations can be used for the optimization of PRRSV and PEDV infection, improved detection and vaccine production. Currently, we are evaluating the effect of spinoculation and combined treatment (polycations and spinoculation) on PRRSV and PEDV entry. Importantly, the effect of the polycations and spinoculation on the PRRSV and PEDV infection has never been studied before.

Characterization of avian pathogenic Escherichia coli (APEC) isolates from clinical cases of colibacillosis from poultry in Québec by NGS

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Avian pathogenic *Escherichia coli* (APEC) causes colibacillosis, which results in morbidity, mortality and economic losses to the poultry industry. Historically, characterization of clinical isolates was done by PCR, slide agglutination and disk diffusion assay. The objective of this work was to characterize APEC isolates using Next Generation-Sequencing (NGS) in order to implement its use in our routine diagnostic microbiology. Around 80 *E. coli* isolates from clinical cases of colibacillosis in poultry from 2014 to 2018 and 30 *E. coli*isolates from healthy chickens were characterized by NGS using an Illumina MiSeq following DNA library preparation with Nextera XT library kit. In silico analysis was performed for virulence genes, O:H serotypes, multilocus sequence types (MLST), phylogenetic grouping, and antimicrobial resistance (AMR) genes. SNP tree and core genome MLST (cgMLST) analysis were also performed. Our results showed that clinical and commensal strains belonging to specific STs and phylogroups tend to cluster together. Better knowledge of APEC from Québec through NGS will facilitate their detection, surveillance and tracking in poultry herds and farms.

Spread of *Escherichia coli* carrying blaCTX-M and blaCMY-2 in Senegalese chicken farms through clones and plasmids

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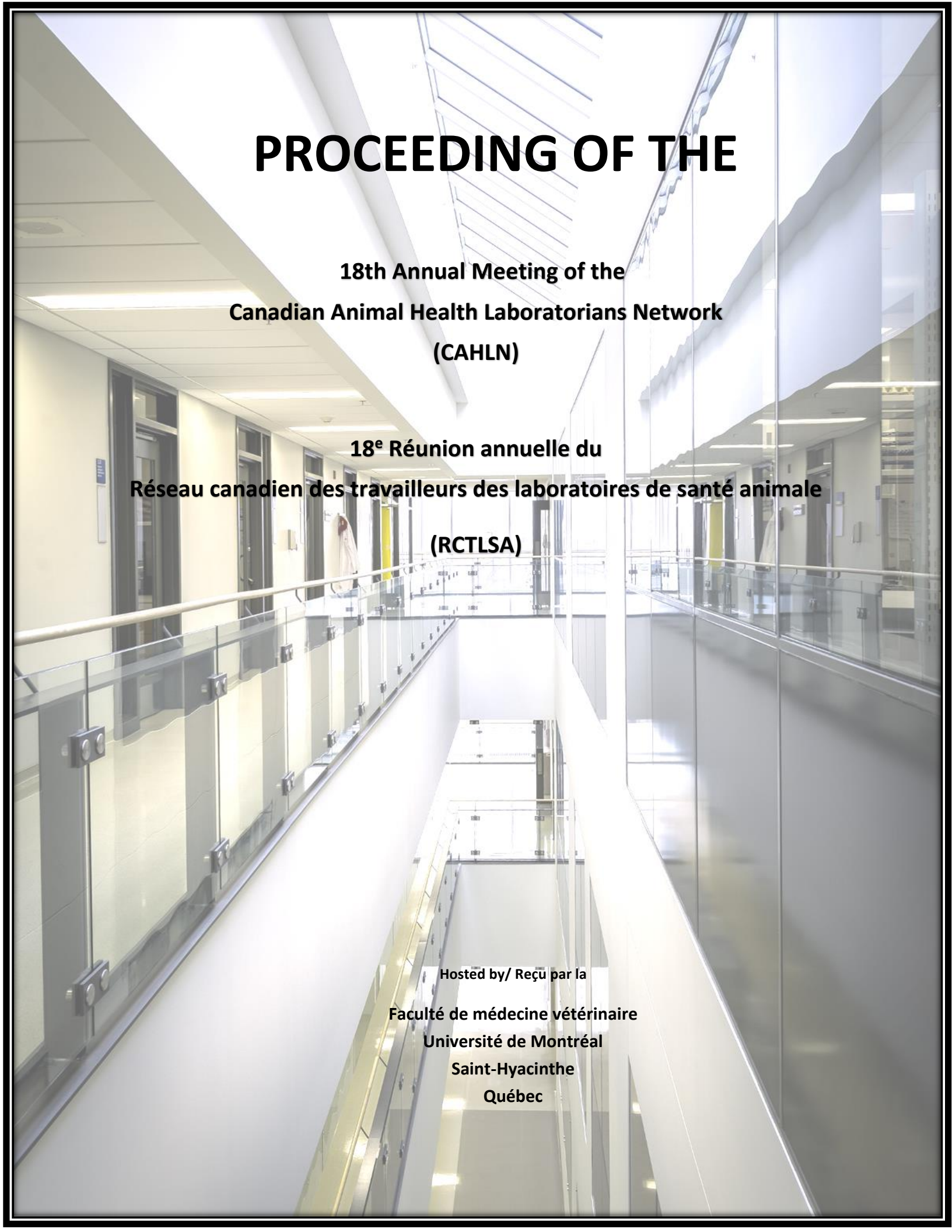
The aim of this study was determine whether healthy chicken farms are a source of dangerous *E. coli* for humans. *E. coli* were isolated from various samples (feces, water and swab carcasses) collected in 32 farms in the region of Dakar and Thiès, Senegal. Generic isolates (n=193) and 130 isolated on selective medium (with ceftriaxone, CTX isolates) were characterized with respect to antimicrobial resistance, clonal relationship and plasmids carrying ESBL/AmpC-genes.

Presumptive ESBL/AmpC-isolates were recovered from 17 (53.1%) farms. In generic collection, 1.5% of isolates were classified presumptive ESBL-producers and 5.7% as presumptive AmpC-producers; this prevalence in CTX collection was 17.0% and 43.1%, respectively. These presumptive ESBL/AmpC-isolates were found in each sample-source. Then, 63 isolates of CTX collection and 128 of generic collection were randomly selected and screened by PCR for bla_{CTX-M} gene encoding resistance to ceftriaxone and bla_{CMY-2}gene mediating resistance against ceftiofur. These isolates were also examined for the four phylogenetic groups A, B1, B2 and D. In the generic collection, 1.0% and 3.0% of isolates, all belonging to phylogroupes A, were positives to bla_{CTX-M} and bla_{CMY-2}, respectively. In CTX collection, these proportions were bla_{CMY-2} (35%) and bla_{CTX-M} (19%); bla_{CMY-2}-producers were of the four phylogroups while bla_{CTX-M}-isolates belonged to phylogenetic groups A, B1 and D. Subtyping of bla_{CTX-M}/bla_{CMY-2} isolates using Pulse Field Gel Electrophoresis (PFGE) showed their high genetic diversity and electroporation reveled that bla_{CTX-M} gene was carried by replicons incI1 and incK while bla_{CMY-2} was located on replicons incI1, incK and incB/O.

Our results demonstrated that although cephalosporins are not used in poultry production in Senegal, *E. coli* recovered from healthy chickens produce ESBL/AmpC. The presence of these ESBL/AmpC-producers may be due to co-resistance following the extensive use of other antimicrobials that share the same resistance plasmids with cephalosporins. The CTX-M and CMY are important extended-spectrum beta lactamases in human medicine and have become a threat to public health. Our findings call for monitoring *E. coli* in healthy chickens in Senegal and regulation of antimicrobial use in poultry. Moreover, it is important to raise awareness of farmers on biosecurity measures since *E. coli*-carrying ESBL/AmpC genes could be transmitted to humans via contaminated carcasses or through direct contact.



PROCEEDING OF THE

18th Annual Meeting of the

Canadian Animal Health Laboratorians Network

(CAHLN)

18e Réunion annuelle du

Réseau canadien des travailleurs des laboratoires de santé animale

(RCTLSA)

Hosted by/ Reçu par la

Faculté de médecine vétérinaire

Université de Montréal

Saint-Hyacinthe

Québec