



**CAHLN /
RCTLSA
2010**



Proceedings
Compte Rendu

**9th Annual Meeting of the
Canadian Animal Health
Laboratorians Network
(CAHLN)**

**9e Congr s Annuel du R seau
Canadien des Travailleurs des
Laboratoires de Sant  Animale
(RCTLSA)**

June 6 – 9, 2010
6 au 9 juin, 2010

Faculty of Veterinary Medicine
University of Calgary
3330 Hospital Drive
Calgary, AB T2N 4N1



CAHLN / RCTLSA 9th Annual Meeting

Local Organizing Committee:

Oscar Illanes, Chair, UCVM
Ophira Charikar, UCVM
Susan Cork, UCVM
Carmen Fuentealba, UCVM
Amy Warren, UCVM
Janet Webb, UCVM

CAHLN / RCTLSA National Steering Committee:

Marie Archambault, Faculté de Médecine Vétérinaire, Université de Montréal
Jim Goltz, Provincial Veterinary Laboratory, NB
Grant Maxie, Animal Health Laboratory, University of Guelph, ON
Anne Muckle, Diagnostic Services, AVC, PEI
Maria Perrone, CFIA, Ottawa, ON
Marc Swendrowski, Manitoba Agriculture, MB

9th Annual Meeting of the Canadian Animal Health Laboratorians Network (CAHLN)

June 6 – 9, 2010

**Faculty of Veterinary Medicine
University of Calgary
3330 Hospital Drive
Calgary, AB T2N 4N1**

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, pathology, immunology, virology, Parasitology, toxicology and molecular biology.

Previous annual meetings have been held in:

2002 – Ottawa (CFIA – OLF)
2003 - Ottawa (CFIA – OLF)
2004 – Guelph (Ontario Veterinary College)
2005 – St-Hyacinthe (Faculté de Médecine Vétérinaire)
2006 - Ottawa (CFIA – OLF)
2007 – Saskatoon (Western College of Veterinary Medicine/Prairie Diagnostic Services)
2008 - Ottawa (CFIA – OLF)
2009 – Charlottetown (AVC – UPEI)
2010 – Calgary (UCVM)

9^e congrès annuel du réseau canadien des travailleurs des laboratoires de santé animale (RCTLSA)

Du 6 au 9 juin, 2010

**Faculty of Veterinary Medicine
University of Calgary
3330 Hospital Drive
Calgary, AB T2N 4N1**

Le RCTLSA a été créé en 2002 dans le but de favoriser l'échange d'information 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 préoccupation 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, en immunologie, en virologie, en parasitologie, en toxicologie et en biologie moléculaire.

Les congrès annuels précédents ont eu lieu à:

2002 – Ottawa (CFIA – OLF)
2003 - Ottawa (CFIA – OLF)
2004 – Guelph (Ontario Veterinary College)
2005 – St-Hyacinthe (Faculté de Médecine Vétérinaire)
2006 - Ottawa (CFIA – OLF)
2007 – Saskatoon (Western College of Veterinary Medicine/Prairie Diagnostic Services)
2008 - Ottawa (CFIA – OLF)
2009 – Charlottetown (AVC – UPEI)
2010 – Calgary (UCVM)

CAHLN / RCTLSA AWARDS

1. CAHLN Laboratorian of the Year – Prix du Diagnosticien du RCTLSA

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

Le Réseau canadien des travailleurs des laboratoires de santé animale (RCTLSA) décerner chaque année une plaque à un des siens, pour sa ou ses contributions à la médecine vétérinaire de laboratoire au Canada. Le comité des récompenses considérera les noms qui seront proposés par tout travailleur canadien de laboratoire. Le candidat peut être un diagnosticien, un éducateur, un chercheur, un mentor de la relève ou n'importe qui du domaine, dont l'apport est remarquable. La récompense est décernée à la fin de la réunion annuelle du Réseau.

Past winners of the CAHLN Laboratorian of The Year Award:

- 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

2. CAHLN Graduate Student Presentation Award

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

Chaque année, une plaque est remise au finissant qui a présenté le meilleur exposé dans le cadre de l'assemblée annuelle du Réseau canadien des travailleurs des laboratoires de santé animale (RCTLSA). Les exposés sont jugés selon l'originalité du sujet, la contribution de l'exposé aux connaissances de base, la compréhension et la présentation de la matière, ainsi que la capacité des finissants de répondre aux questions. La récompense est décernée à la fin de la réunion annuelle du Réseau.

Past winners of the CAHLN Graduate Student Presentation Award:

- 2003 – Sherry Andrews, WCVN, Saskatoon, SK
- 2004 – Noel Harrington, OVC/CFIA, ON
- 2005 – Guillaume Bruant, FMV, St. Hyacinthe, QC
- 2006 – Yuanmu Fang, WCVN, Saskatoon, SK
- 2007 – Kathi Ellis, WCVN, Saskatoon, SK
- 2008 – Angela Catford, OVC, Guelph, ON
- 2009 – Raphael Vanderstichel, AVC, Charlottetown, PEI



**CAHLN /
RCTLSA
2010**



**A very special thank you is extended to all
of our sponsors for their contributions to
the CAHLN/RCTLSA 2010 Annual Meeting!**

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**CAHLN / RCTLSA and Special Symposium Programs
Sunday, June 6, 2010**

CAVP-ACPV Annual Meeting

8:00 AM – 5:00 PM Annual General Meeting
TRW 2E23, Teaching Research & Wellness Building

CAHLN / RCTLSA Annual Meeting

Overall Theme: “One health approach for the detection and monitoring of transmissible spongiform encephalopathies”

5:00 – 6:00 PM Registration

5:30 – 6:30 PM Opening Reception for the 2010 CAHLN Meeting - Cash bar available

Registration and reception will take place at the Health Sciences Centre (HSC) atrium and mall

6:30 – 7:30 PM Invited Speaker: Dr. Josephine Smart: “*The Impact of BSE Policies on Animal Management in Alberta*”

Dr. Smart is a Professor in the Department of Anthropology, University of Calgary, Alberta.

CAHLN / RCTLSA 9th Annual Meeting
Opening Session
Monday, June 7, 2010
8:30 AM – 12:30 PM
Theatre 1, Health Sciences Center

English/ French translation services will be provided during this day thanks to the generous support of the Canadian Food Inspection Agency.

7:30 – 8:30 AM Morning Tea/ Coffee and registration

8:30 - 9:00 AM Official welcome/ opening remarks: Chair, local organizing committee - 5 minutes. Welcome from the Dean, Dr. Alastair Cribb – 10 minutes. Welcome from Dr. Gerald Hauer, Chief Provincial Veterinarian, Alberta Agriculture and Rural Development – 15 minutes.

Moderator: **Dr. Jan Bystrom**, Food Safety and Animal Health Division, Alberta Agriculture & Rural Development, Airdrie Agriculture Centre, Alberta

9:00 – 10:00 AM Invited Speaker: Dr. Stefanie Czub: *“BSE – What else is new?”*

Dr. Czub is the Head of the CFIA National and OEI BSE Reference Laboratory located in Lethbridge, Alberta. Dr. Czub is also Adjunct Faculty at the University of Calgary, Faculty of Veterinary Medicine (UCVM).

10:00 - 10:45 AM Tea/Coffee Break – Posters – Networking

10:45 – 11:45 AM Invited Speaker: Dr. Aru Balachandran: *“Prion diseases of small ruminants and cervids in Canada (1990-2010): an overview of epidemiology, prevalence, diagnosis and approaches to surveillance and control”*

Dr. Balachandran is the Head, National and OIE Reference Laboratory for Scrapie and CWD, Animal Disease Research Institute, Canadian Food Inspection Agency, Ottawa, Ontario.

11:45 AM -12:30 PM Invited Speaker: Dr. Catherine Graham: *“Study of the Pathogenesis of Chronic Wasting Disease (CWD) in captive elk”*

Dr. Graham is a Veterinary Pathologist at the CFIA National and OEI BSE Reference Laboratory located in Lethbridge, Alberta.

12:30 - 1:30 PM Lunch – Health Sciences Center Atrium

1:00 - 1:45 PM *Business Meeting – CAHLN / RCTLSA (Open) - Dr. Grant Maxie, Theatre 1*

CAHLN / RCTLSA 9th Annual Meeting

Monday, June 7, 2010

1:45 PM – 5:45 PM

Theatre 1, Health Sciences Center

Moderator: **Dr. Jim Goltz**, Veterinary Laboratory and Pathology Services, Agricultural Research Station, Fredericton, New Brunswick.

1:45 - 2:45 PM Invited Speaker: Dr. Trent Bollinger: *“CWD in wild cervids: surveillance, research & public policy”*

Dr. Bollinger is Associate Professor at the Department of Veterinary Pathology, WCVM, University of Saskatchewan, Saskatchewan.

2:45 - 3:05 PM *“Evaluation of the analytical sensitivity of tests used for BSE surveillance and BSE confirmation”* Presenter: John Gray, National and OEI BSE Reference Laboratory, Canadian Food Inspection Agency, Lethbridge, Alberta

3:05 -3:25 PM *“Development of electronic microarray for the simultaneous detection and typing of avian influenza and Newcastle disease viruses”* Presenter: Sam Ohene-Adjei, Canadian Food Inspection Agency, Lethbridge Laboratory, Lethbridge, Alberta

3:25-3:45 PM *“Detection of bovine high consequence agents on an electronic microarray platform”* Presenter: Kimberly Burton Hughes, Canadian Food Inspection Agency, Lethbridge Laboratory, Lethbridge, Alberta

3:45-4:30 PM Tea/Coffee Break – Posters – Networking

4:30-5:15 PM Invited Speaker: Dr. Ellen Goddard: *“Canadian Public Awareness of, and concerns about TSEs: BSE and Chronic Wasting Disease”*

Dr. Goddard is a Professor in the Department of Rural Economy, University of Alberta, Alberta.

5:15-5:45 PM Tea/Coffee Break – Posters – Networking

6:00 - 9:00 PM *CAHSN Steering Committee Meeting - Dr. Soren Alexandersen (CFIA), Dean’s Boardroom, 2nd Floor, TRW Building*

CAHLN / RCTLSA 9th Annual Meeting

Tuesday, June 8, 2010

8:30 AM – 11:45 AM

Theatre 1, Health Sciences Center

Moderator: **Dr. Estela Cornaglia**, Diagnostic Services, Faculty of Veterinary Medicine, University of Montreal, Saint-Hyacinthe, Québec.

- 7:30 - 8:30 AM Morning Tea/Coffee - Networking
- 8:30 - 8:50 AM *“Strong and weak points of two software applications to manage documentation, sample conservation and metrology”* Presenter: Véronique Boyer, Diagnostic Services, Faculty of Veterinary Medicine, University of Montreal, Québec
- 8:50 - 9:10 AM *“Fore-CAN Project: Application of foresight to animal health emergency management”* Presenter: Shane Renwick, Canadian Food Inspection Agency, Ottawa, Ontario
- 9:10 - 9:30 AM *“A survey of the emergency preparedness status of Canadian veterinary diagnostic laboratories”* Presenter: Dr. Maria T. Spinato, Animal Health Laboratory, University of Guelph, Ontario
- 9:30 - 10:00 AM *“Chronic Wasting Disease (CWD) surveillance in moose: Collaboration with First Nations Community Hunters in Alberta”* Presenter: Carmen Fuentealba, Department of Ecosystems & Public Health, University of Calgary – Faculty of Veterinary Medicine (UCVM), Calgary, Alberta
- 10:00 - 10:30 AM Tea/Coffee Break – Posters – Networking
- 10:30 - 11:15 AM** ***Panel discussion: “TSE - future directions and challenges”***
Moderator: Dr. Kevin Keough, Executive Director of the Alberta Prion Research Institute (APRI)
Panelists: Invited Speakers
- 11:15 - 11:45 AM *“Research activities in ecosystem health & toxicology”* Presenter: Judit Smits, Department of Ecosystems & Public Health, University of Calgary – Faculty of Veterinary Medicine (UCVM), Calgary, Alberta
- 11:45 AM - 1:00 PM Lunch – Health Sciences Center Atrium

CAHLN / RCTLSA 9th Annual Meeting

Tuesday, June 8, 2010

1:00 PM – 4:10 PM

Theatre 1, Health Sciences Center

Moderator : **Dr. Carmencita Yason**, Diagnostic Services, Atlantic Veterinary College, University of Prince Edward Island, Charlottetown, PEI.

- 1:00 - 1:40 PM *“Which quality program is best for your lab?”* Presenter: Grant Maxie, Animal Health Laboratory, Laboratory Services Division, University of Guelph, Guelph, Ontario
- 1:40 - 2:00 PM *“Rapid identification of bovine mastitis pathogens by high resolution melt analysis of 16S rDNA sequences”* Presenter: Praseeda Ajitkumar, Department of Production Animal Health, University of Calgary – Faculty of Veterinary Medicine (UCVM), Calgary, Alberta
- 2:00 - 2:30 PM *“Equine Inflammatory Airway Disease (IAD): Epidemiology, diagnosis and treatment of this common Th2 type inflammatory condition of horses”*
Presenter: Renaud Leguilette, Department of Veterinary Clinic & Diagnostic Sciences, University of Calgary – Faculty of Veterinary Medicine (UCVM), Calgary, Alberta
- 2:30 - 3:00 PM Coffee Break – Networking

Moderator: **Ms. Marilyn Jonas**, Prairie Diagnostic Services, University of Saskatchewan, Saskatoon, SK

- 3:00 - 3:20 PM *“Morphologic and molecular pathogenesis study of lesions in condemned porcine kidneys”* Presenter: Claudia Benavente, Institute of Microbiology & Infectious Diseases, Faculty of Medicine, University of Calgary, Calgary, Alberta
- 3:20-3:40 PM *“Development of a new immunochromatographic strip assay to detect the infection caused by PRRSV in pigs”* Presenter: Maamar Achacha, Arivac Inc., St-Hyacinthe, Québec.
- 3:40 - 4:10 PM *“Johne’s disease in cattle and Crohn’s disease in humans – linked diseases?”*
Presenter: Herman Barkema, Department of Production Animal Health, University of Calgary – Faculty of Veterinary Medicine (UCVM), Calgary, Alberta.
- 4:35 - 5:00 PM Bus trip to the Clinical Skills Building (CSB), Spy Hill Campus, University of Calgary – Faculty of Veterinary Medicine (UCVM)

5:00 - 6:00 PM Tea/Coffee – Networking - CSB tours

6:00 - 8:00 PM *Dinner at the CSB*

8:00 PM *Bus returns to the Foothills Campus and to Best Western Village Park Inn*

CAHLN / RCTLSA 9th Annual Meeting

Wednesday, June 9, 2010

9:00 AM – 12:00 PM

Theatre 1, Health Sciences Center

Moderator: TBA

- 8:00 - 9:00 AM Morning Tea/ Coffee - Networking
- 9:00 - 9:20 AM *“Use of bacterial antimicrobial resistance gene microarray for the identification of Staphylococcus aureus resistance”* Presenter: Philippe Garneau, Centre de Recherché en Infectiologie Porcine (CRIP), Faculté de Médecine Vétérinaire, Université de Montréal, Saint-Hyacinthe, Québec
- 9:20 - 9:40 AM *“A scheme for comprehensive typing of Mycobacterium avium subspecies paratuberculosis strains using real-time high resolution melting”* Presenter: Joel David, Department of Production Animal Health, University of Calgary – Faculty of Veterinary Medicine (UCVM), Calgary, Alberta
- 9:40 - 10:00 AM *“Detection of bovine lymphotropic herpesvirus DNA in tissues of a bovine aborted fetus in Québec”* Presenter: Carl A. Gagnon, Service de Diagnostic, Centre de Recherché en Infectiologie Porcine (CRIP), Groupe de Recherche sur les Maladies Infectieuses du Porc (GREMIP), Faculté de Médecine Vétérinaire, Université de Montréal, Saint-Hyacinthe, Québec
- 10:00 - 10:30 AM Coffee Break-Networking
- 10:30 - 11:00 AM *“Overview of Diagnostic Services at UCVM – challenges and future directions”* Presenter: Oscar Illanes, Department of Production Animal Health, University of Calgary – Faculty of Veterinary Medicine (UCVM), Calgary, Alberta
- 11:00 - 11:20 AM *“Progressive juvenile-onset myeloencephalopathy in Angus cattle”* Presenter: Oscar Illanes, Department of Production Animal Health, University of Calgary – Faculty of Veterinary Medicine (UCVM), Calgary, Alberta
- 11:20 - 11:40 AM** ***Presentation of the Graduate Student Award and Laboratorian of the Year Award - CAHLN/ RCTLSA National Steering Committee***
- 11:40 - 11:50 AM** ***Closing Remarks***
- 12:00 –1:00 PM** ***Lunch***

CAHLN / RCTLSA 9th Annual Meeting
Sunday, June 6, 2010
Theatre 1, Health Sciences Center

		Page
6:30 – 7:30 PM	<u>Invited Speaker</u> : Dr. Josephine Smart: <i>“The Impact of BSE Policies on Animal Management in Alberta”</i>	14

Dr. Smart is a Professor in the Department of Anthropology, University of Calgary, Alberta.

The Impact of BSE Policies on Animal Management in Alberta

Josephine Smart¹

Using in-depth ethnographic interview data collected in selected farming communities in Alberta from 2006 to 2008, and document analysis of the myriad of policy responses to BSE in Canada since 2003, this paper will address an important question of how the BSE policy responses may have unintended negative consequences on animal management at the farm level and food safety in rural Alberta. The key BSE policy responses addressed in this paper are the enhanced feed ban (effective July 2007), and the Alberta Livestock and Meat Agency (ALMA) created in June 2008. Several key observations are made in this paper:

1. While these various national and provincial BSE policy responses are created to control the spread of BSE through traceability, MBM ban in feeds, SRMs removal and other best practices, they have also created extra costs and stress to the farmers which can affect their effectiveness in farm management and their economic and social well being.
2. One of the unintended outcomes of the enhanced feed ban regarding SRM removal and transport protocol is the elimination of small operators in rural communities who run small meat shops and butchering facilities and are without the economic resources to meet the new infrastructure guidelines in accordance with the SRM and OTM (over thirty months) biosecurity requirements. This elimination of rural meat shops and butchering facilities forces many farmers to revert to home butchering in violation of the existing by-laws. This reversion to home butchering is done for economic reasons, i.e. it costs increasingly more time and money to truck your few animals to one of the few butchering facilities still in operation in rural Alberta. There are food safety implications that are still too early to assess.
3. The disposal of dead cattle on the farm has become more complex and expensive under the new guidelines. For farmers who are seeing a downturn in their income due to the strong Canadian dollar in 2009, the decline in export due to COOL (US) and other factors, and the decline on off-farm income since the onset of an economic recession in the fall of 2008, the disposal of dead animals on the farm has become an increasingly attractive option despite the immediate and long term risks of prion contamination at the disposal site.
4. The continuing decline in the value of live stock and beef products has strengthened the already existing response among many farmers to reduce the use of veterinary services as a cost saving strategy. While farmers have always taken on a large share of the health management and medication of their farm animals, their inclination to undertake major surgery on animals and other animal disease management without professional intervention is an issue that deserves further investigation.

¹ Department of Anthropology, University of Calgary

CAHLN / RCTLSA 9th Annual Meeting
Opening Session

Monday, June 7, 2010

8:30 AM – 12:30 PM

Theatre 1, Health Sciences Center

Theme: “One health approach for the detection and monitoring of transmissible spongiform encephalopathies”

Page

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Moderator: **Dr. Jan Bystrom**, Food Safety and Animal Health Division, Alberta
Agriculture & Rural Development, Airdrie Agriculture Centre, Alberta

9:00 – 10:00 AM Invited Speaker: Dr. Stefanie Czub: “*BSE – What else is new?*” 16

Dr. Czub is the Head of the CFIA National and OEI BSE Reference Laboratory located in Lethbridge, Alberta. Dr. Czub is also Adjunct Faculty at the University of Calgary, Faculty of Veterinary Medicine (UCVM).

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Dr. Balachandran is the Head, National and OIE Reference Laboratory for Scrapie and CWD, Animal Disease Research Institute, Canadian Food Inspection Agency, Ottawa, Ontario.

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Dr. Graham is a Veterinary Pathologist at the CFIA National and OEI BSE Reference Laboratory located in Lethbridge, Alberta.

12:30 - 1:30 PM Lunch – Health Sciences Center Atrium

1:00 - 1:45 PM *Business Meeting – CAHLN / RCTLSA (Open) - Dr. Grant Maxie, Theatre 1*

BSE - What Else is New?

Stefanie Czub¹

Prion diseases or transmissible spongiform encephalopathies are neurodegenerative diseases that affect both human and animals. They can be transmitted between mammals by inoculation with, or in some cases by dietary exposure to infected tissues. These diseases are closely connected with a host glycoprotein which has been called prion protein (PrP). Inherited forms of prion diseases are associated with coding mutations in this gene. The key event which triggers the development in all prion diseases is the post-translational, non-covalent transition of the normal isoform (PrP^c) to the infectious form (PrP^{Sc}). The formation of PrP^{Sc}, which is the major and maybe the only component of the infectious prion particle, leads to a slowly progressing neurological illness with extended incubation times, dementia and ataxia as the most prominent signs and an invariably fatal outcome. The classical neuropathological changes are limited to the central nervous system and consist of spongiform degeneration, amyloid plaques, astrocytic gliosis and nerve cell loss by apoptosis. Epidemiological, pathological and molecular data suggest that Bovine Spongiform Encephalopathy (BSE) is related to variant Creutzfeldt-Jakob disease (vCJD) of humans.

Canada has a suite of robust BSE control measures in place and their assessment by the OIE has resulted in the recognition as a controlled risk country (May 2007). The OIE categorization process is based on an evaluation of the comprehensive surveillance, mitigation and eradication measures implemented. In 1990, the Canadian Food Inspection Agency (CFIA) named BSE a reportable disease, followed by the creation of a surveillance program in 1992 in which high-risk cattle are tested for the disease. As an additional layer of protection, Specific Risk materials (SRMs) are removed from all cattle slaughtered for human consumption and are banned from all animal feed, pet foods and fertilizers (July 12, 2007). With these risk mitigation measures in place, it is expected to detect a small number of cases over the next 10 years as Canada progresses towards eliminating BSE from the national cattle herd. To monitor the effectiveness of the safeguards, a national surveillance program had been implemented in 1992. The program targets cattle most at risk and is delivered through the BSE laboratories network which is a collaboration of federal and provincial governments, universities and private veterinary practitioners coordinated by the BSE Reference Laboratory (RL) in Lethbridge/Alberta.

This presentation provides an overview of the national & international BSE situation (including the significance of atypical BSE), of the Canadian BSE safeguards and of the research performed at the Canadian & OIE Reference Laboratories for BSE.

¹Canadian & OIE Reference Laboratories for BSE, CFIA Lethbridge Laboratory/University of Calgary, Lethbridge, Alberta

Prion Diseases of Small Ruminants and Cervids in Canada (1990-2010): an Overview of Epidemiology, Prevalence, Diagnosis and Approaches to Surveillance and Control

Dr. Aru Balachandran¹

Scrapie in sheep and goats is the longest known Transmissible Spongiform Encephalopathy (TSE) or Prion disease and has been a reportable disease in Canada since 1945. Chronic wasting disease (CWD) has recently emerged in North America as an important TSE of captive and free-ranging cervids (species in the deer family, elk and moose). The natural history of CWD is incompletely understood, but it differs from scrapie and bovine spongiform encephalopathy (BSE) by virtue of its occurrence in non domestic and free-ranging species. CWD has many features in common with scrapie, including early widespread distribution of abnormal prion protein (PrP^d) in lymphoid tissues, with later involvement of central nervous system (CNS) and peripheral tissues. There are marked similarities in the clinical presentation and pathological lesions and some similarities in the epidemiology. Early accumulation of infectivity in alimentary tract-associated lymphoid tissues during incubation suggests agent shedding in feces or saliva as plausible transmission routes. CWD like scrapie is laterally transmitted and environmental contamination may play an important role in local maintenance of the disease. A range of risk factors at individual animal level (such as genotype, age, gender) as well as the flock/herd level (such as the size, husbandry practices, contact with other farms etc.) have been associated with the level of infection and clinical disease. Over the past decade diagnostic tests have been improved, allowing diagnosis early in the incubation period, long before the appearance of clinical disease. Surveillance efforts for CWD in captive and free-ranging cervids will continue in concert with similar activities for scrapie and BSE. Programs have been developed and instituted by wildlife management authorities for free-ranging deer and elk and for the domestic sheep and cervids by provincial and federal agencies.

Although CWD can be transmitted by intracerebral inoculation to cattle, sheep, and goats, completed and ongoing studies have not demonstrated that domestic livestock are susceptible via oral exposure, the presumed natural route of exposure to TSEs.

Experimental studies have shown that BSE is transmissible to sheep by the oral route and affected sheep have tissue distribution of infectivity and pathological changes similar to those of scrapie affected sheep. Following the introduction of active monitoring of small ruminants for BSE in 2002, a novel and distinct form of scrapie phenotype was identified in Europe and North America including 7 in Canada so far. These cases, called atypical scrapie or Nor98, diagnosed mainly by using the rapid ELISA test, seemed to differ from classical scrapie in their brain distribution of PrP^d, their epidemiology, and their occurrence in sheep of PrP genotypes resistant to developing clinical scrapie.

Unlike BSE, there is no epidemiological, clinical or experimental evidence to date that connects scrapie to human prion diseases. However, the consequences of CWD and other sheep TSEs and their spread yet to be determined; moreover, public health implications remain a question of intense interest.

¹National and OIE Reference Laboratory for scrapie and CWD, Canadian Food Inspection Agency

Study of the Pathogenesis of Chronic Wasting Disease in Captive Elk

Catherine Graham¹, Stefanie Czub¹, Tammy Pickles¹, Martin Kaatz¹, Aru Balachandran², Gary Wobeser³,
Elemir Simko³

Chronic wasting disease (CWD) of farmed and feral cervids is a transmissible spongiform encephalopathy (TSE) of increasing prevalence in North America. In naturally infected Rocky mountain elk (*Cervus elaphus nelsoni*) CWD infectivity has been demonstrated mainly in the brain and lymphoreticular tissues. Information on the pathogenesis of this disease, especially the distribution of PrP^{CWD} in infected elk at various stages of the incubation is incomplete. To understand and define the specified risk materials in elk CWD, we inoculated 4 month old captive female elk (n= 23 infected, 7 control), belonging to 132MM and 132LM genotypes, via the oral route with brain homogenate from elk diagnosed with clinical CWD (infected) or negative for CWD (control) and the disease progression was monitored at regular intervals. Tissue distribution of PrP^{CWD} was evaluated by immunohistochemistry using monoclonal antibody F99. In 132MM genotype elk, the incubation periods were consistent with early onset of clinical disease at 19-21 months post inoculation (PI). Progressive clinical signs characteristic of CWD were observed and the animals were euthanized at severe end stage. Onset of disease in 132LM genotype animal was delayed (23 months) and the clinical signs were subtle. In 132MM group, PrP^{CWD} was detected within neurons in the myenteric plexus of the ileum and focally in the ventrolateral portion of the dorsal motor nucleus of the vagal nerve within the medulla oblongata at approximately 300 days PI. No detectable staining was seen in the 132LM animal euthanized at the same time point. In 132MM group progressive distribution of PrP^{CWD} was observed in the alimentary tract and lymphoid tissues from day 400PI onwards, and animals euthanized with severe clinical disease had widespread positive staining throughout the central nervous system, gastrointestinal tract, adrenal medulla and most lymphoid tissues examined. The single 132LM animal euthanized at clinical disease had positive staining with a similar distribution but the intensity was significantly less. Comparative analyses of infectivity distribution and genetics will be presented and the implications discussed.

¹Canadian Food Inspection Agency, Lethbridge Laboratory, Lethbridge, Alberta, Canada

²National Reference Laboratory for Scrapie and CWD, Canadian Food Inspection Agency, Ottawa, Ontario, Canada

³Department of Veterinary Pathology, University of Saskatchewan, Saskatoon, Saskatchewan, Canada

CAHLN / RCTLSA 9th Annual Meeting

Monday, June 7, 2010

1:45 PM – 5:45 PM

Theatre 1, Health Sciences Center

Moderator: **Dr. Jim Goltz**, Veterinary Laboratory and Pathology Services, Agricultural Research Station, Fredericton, New Brunswick.

		Page
1:45 - 2:45 PM	<u>Invited Speaker</u> : Dr. Trent Bollinger: <i>“CWD in wild cervids: surveillance, research & public policy”</i>	20
	Dr. Bollinger is Associate Professor at the Department of Veterinary Pathology, WCVN, University of Saskatchewan, Saskatchewan.	
2:45 - 3:05 PM	<i>“Evaluation of the analytical sensitivity of tests used for BSE surveillance and BSE confirmation”</i> <u>Presenter</u> : John Gray, National and OEI BSE Reference Laboratory, Canadian Food Inspection Agency, Lethbridge, Alberta	21
3:05 -3:25 PM	<i>“Development of electronic microarray for the simultaneous detection and typing of avian influenza and Newcastle disease viruses”</i> <u>Presenter</u> : Sam Ohene-Adjei, Canadian Food Inspection Agency, Lethbridge Laboratory, Lethbridge, Alberta	22
3:25-3:45 PM	<i>“Detection of bovine high consequence agents on an electronic microarray platform”</i> <u>Presenter</u> : Kimberly Burton Hughes, Canadian Food Inspection Agency, Lethbridge Laboratory, Lethbridge, Alberta	23
3:45-4:30 PM	Tea/Coffee Break – Posters – Networking	
4:30-5:15 PM	<u>Invited Speaker</u> : Dr. Ellen Goddard: <i>“Canadian Public Awareness of, and concerns about TSEs: BSE and Chronic Wasting Disease”</i>	24
	Dr. Goddard is a Professor in the Department of Rural Economy, University of Alberta, Alberta.	
5:15-5:45 PM	Tea/Coffee Break – Posters – Networking	
6:00 - 9:00 PM	<i>CAHSN Steering Committee Meeting - Dr. Soren Alexandersen (CFIA), Dean’s Boardroom, 2nd Floor, TRW Building</i>	

CWD in Wild Cervids: Surveillance, Research & Public Policy

Trent K. Bollinger¹

Since CWD was first detected in farmed elk in Saskatchewan in 1996, Saskatchewan Environment and the Canadian Cooperative Wildlife Health Centre have undertaken a surveillance program for CWD in wild cervids. First detected in 2000 in a mule deer near the Alberta border south of Lloydminster the disease is now considered endemic over large areas of western Saskatchewan and into eastern Alberta. Although the prevalence in most wildlife management zones remains low there is at least 1 focus along the South Saskatchewan River where the prevalence is over 15%. Over many areas of the province samples sizes are insufficient to say with certainty the disease is not present at a low prevalence. CWD control programs to date have been ineffective and since there are no known impediments to the spread of CWD, it is anticipated the disease will spread throughout Canada and the US with uncertain implications.

Since 2006, with funding from PrioNet Canada and the Alberta Prion Research Institute, we have undertaken research, using both population genetics and radio-telemetry, to attempt to understand factors which affect transmission and geographic spread of CWD in wild cervids. The findings to date will be reviewed.

¹Department of Veterinary Pathology, WCVI, University of Saskatchewan, Canada

Evaluation of the Analytical Sensitivity of Tests Used for BSE Surveillance and BSE Confirmation

John Gray¹, Sandor Dudas¹, Stefanie Czub¹

Following the outbreak of *bovine spongiform encephalopathy* (BSE), surveillance programs have been employed in numerous countries to monitor BSE prevalence and to protect animal and human health. Since 1999, the European Commission (EC) authorized the evaluation and approval of 20 molecular based tests for the rapid detection of the pathological prion protein (PrP^{Sc}) in BSE infection. The diagnostic sensitivity, convenience, and speed of these tests have made molecular diagnostics the preferred method for BSE surveillance. The aim of this study was to determine the analytical sensitivity of 4 commercially available BSE rapid-test kits, including the Prionics® Check-PrioSTRIP™, the Prionics® Check WESTERN™, the BioRad® TeSeE™ ELISA, and the IDEXX® HerdChek™ EIA. Performances of these tests were then compared to 2 confirmatory tests, including the BioRad® TeSeE™ Western Blot and the modified *Scrapie Associated Fibrils (SAF)/OIE Immunoblot*.

A single 50% w/v homogenate was made from experimentally generated C-type BSE bovine brain tissues in ddH₂O. The homogenate was diluted through a background of BSE negative homogenate. Masses of both positive and negative tissues in each dilution were calculated to maintain the appropriate tissue amounts for each test platform. Specific concentrated homogenization buffer was added accordingly to maintain the correct buffer condition for each test. ELISA-based tests were evaluated as per their respective software/detection platforms, and blot-protocols were evaluated by manual measurements of blot density. Detection limits were determined by fitted curves intersecting the manufactures' positive/negative criteria.

As expected, the highest analytical sensitivity was displayed by the confirmatory SAF Immunoblot, followed by the HerdChek™ EIA, TeSeE™ Western, TeSeE™ ELISA, Check WESTERN, and Check PrioSTRIP, respectively. Although the most sensitive and least sensitive rapid tests were separated by 2 logs in analytical sensitivity, all rapid tests appear suitable for targeted BSE surveillance programs as implemented in Canada.

¹Canadian & OIE Reference Laboratories for BSE, Canadian Food Inspection Agency, Lethbridge, Alberta

Development of Electronic Microarray for the Simultaneous Detection and Typing of Avian Influenza and Newcastle Disease Viruses

Sam Ohene-Adjei¹, Anne Beeston¹, Dirk Dereg¹, Tara Furukawa-Stoffer¹, Kristen Hahn¹, Kimberley Burton Hughes¹, Oliver Lung¹, Dalibor Hodko², John Pasick³, Yohannes Berhane³

Avian influenza and Newcastle disease are two major economically important viral diseases that affect poultry. Sensitive, specific and rapid assay for detection and typing of these viruses is essential in facilitating diagnosis and response in the event of an outbreak. In this study, electronic microarray assays were developed for the simultaneous detection of avian influenza viruses (AIV) and detection and pathotyping Newcastle disease viruses (NDV). The AIV and NDV matrix (M) genes and the NDV fusion (F) gene were amplified by a multiplex reverse-transcriptase PCR and hybridized to an electronic microarray with virus-specific and NDV pathotype differentiating probes. Specificity of the assays was tested using 43 AIV strains representing all 16 H-types and 9 N-types, and 22 NDV strains. Sensitivity determination and validation of the assays were performed using ten-fold serially diluted viruses with known titre (10^5 TCID₅₀) and clinical samples respectively. The results indicate that the microarray could be used to simultaneously detect and differentiate AIV and NDV infections, and differentiate between high and low NDV pathotypes. The limit of detection was strain dependent and ranged from 100-1000 TCID₅₀.

¹Canadian Food Inspection Agency, Lethbridge Laboratory, Lethbridge, Alberta, Canada

²Nexogen Inc., San Diego, California, USA

³National Centre for Foreign Animal Diseases, Canadian Food Inspection Agency, Winnipeg, Manitoba, Canada

Detection of Bovine High Consequence Agents on an Electronic Microarray Platform

Oliver Lung¹, Tara Furukawa-Stoffer¹, Kristen Hahn¹, Kimberley Burton Hughes¹, Sam Ohene-Adjei¹, Anne Beeston¹, Dirk Dereg¹, Dalibor Hodko², John Pasick³, Yohannes Berhane³, Donald King⁴, Scott Reid⁴

Two assays for detection and typing of high consequence pathogens in cattle have been developed on the NanoChip 400 platinum electronic microarray. The high consequence pathogen assay is performed as two separate components. One component simultaneously detects and types foot-and-mouth disease (FMD) virus to the serotype level, and the second component detects viruses that cause Vesicular Stomatitis, Exotic Malignant Catarrhal Fever, Rinderpest, Blue Tongue, Bovine Viral Diarrhea, Infectious Bovine Rhinotracheitis and the Parapox Complex. The second assay, the FMD strain identification assay, attempts to identify FMD viruses to the strain level based on several highly variable loci in the genome of the virus. Twenty-three strains representing all FMD serotypes were used for selection of serotype-specific probes and for validation of the FMD PCR. Thirty-eight additional viral strains were used to develop a multiplex PCR for the 7 other exotic and differential target viruses. All available strains were amplified by the multiplex PCR. The high consequence pathogen assay was further validated with clinical and spiked samples. The assays are currently being transferred to a sample-to-answer system that integrates sample preparation, PCR, and a carbon electronic microarray.

¹Canadian Food Inspection Agency, Lethbridge Laboratory, Lethbridge, Alberta, Canada

²Nexogen Inc., San Diego, California, USA

³National Centre for Foreign Animal Diseases, Canadian Food Inspection Agency, Winnipeg, Manitoba, Canada

⁴Institute for Animal Health (IAH), Pirbright, Surrey, United Kingdom

Canadian Public Awareness of and Concerns About TSEs: BSE and Chronic Wasting Disease

Ellen Goddard¹

BSE and Chronic Wasting Disease (CWD) have had very different effects on Canadian consumers, producers and communities. These different effects have been related to differences in the scale of the animal disease outbreaks, differences in the size of the industries affected, the fact that CWD affects both wild and farmed animals and differences in consumption of meats from the two industries and from hunting. Two national general population surveys were conducted in 2009 (1500 respondents each) to assess Canadian's knowledge, awareness and concerns about BSE and CWD as compared to other food/health related issues. These two surveys are somewhat comparable to a survey conducted in Canada in 2006. Risk perceptions associated with venison (meat from deer, elk or moose) are statistically significantly higher than those for beef and risk attitudes (willingness to accept the risk of eating venison) much lower. This would follow from the limited experiences Canadians have of eating venison. Canadians do not see either BSE or CWD in wild and farmed deer and elk, as potential risks to human health, ranking them as more risky than GM foods but much less than all other real food safety issues (E. coli, listeriosis, allergens etc.) and perceived food safety issues such as BSE. In both beef and farmed cervid industries, steps have been taken on animal testing (all animals in the cervid industry, a sample in the beef industry) and are evolving on traceability issues (cervid industry recently received a grant from the federal government while traceability is well underway in Canada from birth to slaughter but not yet from birth to final consumer). Consumer interest in these two policy initiatives and willingness to pay for these attributes in their meat purchases are compared for the two industries. In spite of the fact that no Canadians have been made ill from either of these animal diseases, there is significant interest in the Canadian population for more information and for more testing of animals in these two industries. These results are compared across provinces and across rural urban communities.

¹Department of Rural Economy, University of Alberta, Edmonton, Alberta

CAHLN / RCTLSA 9th Annual Meeting

Tuesday, June 8, 2010

8:30 AM – 11:45 AM

Theatre 1, Health Sciences Center

Moderator: **Dr. Estela Cornaglia**, Diagnostic Services, Faculty of Veterinary Medicine, University of Montreal, Saint-Hyacinthe, Québec.

		Page
7:30 - 8:30 AM	Morning Tea/Coffee - Networking	
8:30 - 8:50 AM	<i>“Strong and weak points of two software applications to manage documentation, sample conservation and metrology”</i> <u>Presenter</u> : Véronique Boyer, Diagnostic Services, Faculty of Veterinary Medicine, University of Montreal, Québec	26
8:50 - 9:10 AM	<i>“Fore-CAN Project: Application of foresight to animal health emergency management”</i> <u>Presenter</u> : Shane Renwick, Canadian Food Inspection Agency, Ottawa, Ontario	27
9:10 - 9:30 AM	<i>“A survey of the emergency preparedness status of Canadian veterinary diagnostic laboratories”</i> <u>Presenter</u> : Dr. Maria T. Spinato, Animal Health Laboratory, University of Guelph, Ontario	28
9:30 - 10:00 AM	<i>“Chronic Wasting Disease (CWD) surveillance in moose: Collaboration with First Nations Community Hunters in Alberta”</i> <u>Presenter</u> : Carmen Fuentealba, Department of Ecosystems & Public Health, University of Calgary – Faculty of Veterinary Medicine (UCVM), Calgary, Alberta	29
10:00 - 10:30 AM	Tea/Coffee Break – Posters – Networking	
10:30 - 11:15 AM	<i>Panel discussion: “TSE - future directions and challenges”</i> <u>Moderator</u>: Dr. Kevin Keough, Executive Director of the Alberta Prion Research Institute (APRI) <u>Panelists</u>: Invited Speakers	
11:15 - 11:45 AM	<i>“Research activities in ecosystem health & toxicology”</i> <u>Presenter</u> : Judit Smits, Department of Ecosystems & Public Health, University of Calgary – Faculty of Veterinary Medicine (UCVM), Calgary, Alberta	
11:45 AM - 1:00 PM	Lunch – Health Sciences Center Atrium	

Strong and Weak Points of Two Software Applications to Manage Documentation, Sample Conservation and Metrology

Véronique Boyer¹, Estela Cornaglia¹

The biosafety and the quality assurance requirements are continually increasing since September 11, 2001. The Bill C 11, Human Pathogens and Toxins Act, implies a detail following of strains, proteins, nucleic acids in almost laboratories. The good laboratory practices and the laboratory accreditations (AAVLD, ISO/IEC 17025, OIE) become essential for the future of diagnostic laboratories.

Several software are available for compiling daily measurements and meeting several QA needs. The ideal software must comply with national and international standards, must be versatile and priced affordably. The ideal software must also contain the most advanced tools for maintaining traceable metrological measurement standards, analyzing measurement results, optimizing measurement processes and managing measurement decision risks.

We would like to describe strong and weak points of software that we are currently using. «Omnidoc» is a software application for managing documents. It allows following, reviewing and approving all documents, procedures and protocols for the Diagnostic Service. We already have over 400 documents treated by the software.

«Omni-assistant» is a software application for managing conservation (keeping, freezing, destroying, and inventorying) of samples, strains, and bacteria or viral collections. It allows tracking samples using bar codes and manages when samples have to be destroyed. Moreover, the software can also manage the metrology based on different sensors, (freezers, incubators, room temperature) and follows temperatures 24 hours a day. Other options available are the management of complaints and the creation of automatic processes.

¹Diagnostic Services, Faculty of Veterinary Medicine, University of Montreal

Fore-CAN Project: Application of Foresight to Animal Health Emergency Management

Dr. Shane Renwick¹

A threat to animal health could significantly impact Canada's security given the potential for devastating consequences related to public health, economic security, food safety, and the environment. Canada's Animal Health Emergency Management (AHEM) system must have effective capabilities in place to anticipate, prevent, prepare for, respond to and recover from, animal health emergencies.

One potential mechanism to help chart likely futures and inform on key decision-making on investments in capability is Foresight, which can be defined as a set of strategic tools that emphasize a long-term perspective to gain insight on future needs, capabilities and priorities.

The project has been structured around 3 phases of activity:

- **Phase 1:** Planning, Learning and Community Building;
- **Phase 2:** Applying Foresight to Animal Health Emergency Management; and
- **Phase 3:** Developing Priorities.

Involving participation from federal, provincial, academic and industry partners, the objective of Fore-CAN is to explore the use of foresight in guiding future-focused planning within the AHEM environment.

Through this foresight process involving a series of facilitated events, the key capabilities underpinning the vision for the system of the future were identified as:

- Organization and Decision-Making;
- Information and Communications;
- Expertise and Personnel;
- Science and Technology; and
- Policy, Law and Regulation.

With the project currently moving into Phase 3, principles on how these capability supports would function in a complex multi-jurisdictional and multi-sectoral environment are under development, as well as the suggested key priorities for activity directed at achieving them.

Foresight offers the means to consider the long-term perspective when identifying future risks, needs and required capabilities. Consideration of anticipated future outcomes when directing investment can form an invaluable part of the strategic planning process, particularly for organizations concerned with capital expenditures in emergency management and operating in multi-jurisdictional environments.

¹Canadian Food Inspection Agency, Ottawa, Ontario

A Survey of the Emergency Preparedness Status of Canadian Veterinary Diagnostic Laboratories

Maria T. Spinato¹, Vahab Farzan²

Veterinary diagnostic laboratories play a pivotal role in the early and rapid detection of infectious animal diseases that could cause serious illness in animals and people, interrupt food supply chains, or negatively impact the economic viability of livestock industries. Most Canadian laboratories have little experience in managing a sudden and exponential increase in testing demand. This type of emergency event is accompanied by the need for enhanced biosecurity protocols, human resources and logistical challenges, and increased regulatory and media scrutiny. The ability of the laboratory to meet stakeholders' requirements for rapid and accurate diagnostic testing may become strained under these circumstances. Failure to prepare adequately for these contingencies will slow the response to the outbreak, and negatively impact business continuity.

The objective of this study is to investigate the level of emergency preparedness among the Canadian veterinary diagnostic laboratories involved in the surveillance and diagnosis of emerging and foreign animal diseases. A survey was prepared and administered to the member laboratories of the Canadian Animal Health Surveillance Network. Questions were configured to examine important features such as administrative structure, testing capabilities, data management, laboratory biosecurity, and emergency planning. The latter included critical topics such as staff training, sample tracking, and preparations for surge testing and business continuity. The results of this survey provide a benchmark for Canadian animal health laboratories, and have generated an audit protocol that will assist them in refining their emergency management plans.

¹Animal Health Laboratory, University of Guelph, Ontario

²Department of Population Medicine, University of Guelph, Ontario

Chronic Wasting Disease (CWD) Surveillance in Moose: Collaboration with First Nations Community Hunters in Alberta

Carmen Fuentealba¹

This presentation will describe a project developed to address the gap that exists between Aboriginal communities and decision-makers regarding infectious wildlife diseases and their implications for environmental and human health. This multidisciplinary collaborative research is funded by PrioNet Canada and Alberta Prion Research Institute. The overall goal of the project is to explore the implications of Chronic Wasting Disease (CWD) for Aboriginal communities and to exchange information in highly effective and culturally appropriate ways.

This presentation will focus on two specific objectives of the project:

- Assess presence of CWD and other wildlife diseases in hunted deer, elk and moose
- Develop multidisciplinary and culturally appropriate forms of communication in collaboration with communities and stakeholders that better inform risks associated with CWD.

The steps involved in this project include the collection and testing for CWD in tissues from cervids hunted by members of two First Nations communities: Alexis Nakota Sioux Nation and Paul First Nation. Prior to commencing this community-based monitoring program, two information workshops were offered within the reservations to demonstrate tissue collection procedures and to answer questions regarding common conditions detected by the hunters and people involved in meat preparation. Hunters were also provided with sampling kits consisting of labelled plastic bags. Selected tissues and body fluids including the obex of the brain, liver, kidney, spleen, blood, feces, hide, testicles and long bones were collected along with information on any carcass abnormalities detected by the hunter. Photographs were taken to document the process of sample reception at the regional laboratory and how they were sorted for submission to specialized laboratories (histopathology, bacteriology, toxicology, molecular biology, etc).

During the first year of the project 15 brain samples were collected by hunters and submitted directly to the National and OIE Reference Laboratory for Scrapie and CWD Animal Diseases Research Institute, Canadian Food Inspection Agency, Ottawa. CWD was not detected in the samples submitted. Lesions collected by hunters consisted of Hydatid cysts in lungs and liver, abscesses in skin and liver, and cases of *Fascioloides magna* infection in livers.

CWD is a reportable prion disease in Canada and regulated by the Federal government. Although CWD has not been reported in moose in Canada, its potential presence is a cause of concern for aboriginal people. The sequence of events from reception to processing of the samples for detection of PrP^{CWD} in tissues has been documented using photographs and video which will be distributed in electronic and hard-copy format to members of the participating First Nations and will be discussed in the next workshop to be held in the July of 2010. This collaborative approach will facilitate communication between multidisciplinary research teams and will improve our ability to translate knowledge to aboriginal community members that are a crucial component in animal disease surveillance.

¹Department of Ecosystem and Public Health, Faculty of Veterinary Medicine, University of Calgary, Calgary, Alberta

CAHLN / RCTLSA 9th Annual Meeting

Tuesday, June 8, 2010

1:00 PM – 4:10 PM

Theatre 1, Health Sciences Center

Moderator : **Dr. Carmencita Yason**, Diagnostic Services, Atlantic Veterinary College, University of Prince Edward Island, Charlottetown, PEI.

		Page
1:00 - 1:40 PM	<i>“Which quality program is best for your lab?”</i> <u>Presenter</u> : Grant Maxie, Animal Health Laboratory, Laboratory Services Division, University of Guelph, Guelph, Ontario	32
1:40 - 2:00 PM	<i>“Rapid identification of bovine mastitis pathogens by high resolution melt analysis of 16S rDNA sequences”</i> <u>Presenter</u> : Praseeda Ajitkumar, Department of Production Animal Health, University of Calgary – Faculty of Veterinary Medicine (UCVM), Calgary, Alberta	33
2:00 - 2:30 PM	<i>“Equine Inflammatory Airway Disease (IAD): Epidemiology, diagnosis and treatment of this common Th2 type inflammatory condition of horses”</i> <u>Presenter</u> : Renaud Leguilette, Department of Veterinary Clinic & Diagnostic Sciences, University of Calgary – Faculty of Veterinary Medicine (UCVM), Calgary, Alberta	34
2:30 - 3:00 PM	Coffee Break – Networking	

Moderator: **Ms. Marilyn Jonas**, Prairie Diagnostic Services, University of Saskatchewan, Saskatoon, SK

3:00 - 3:20 PM	<i>“Morphologic and molecular pathogenesis study of lesions in condemned porcine kidneys”</i> <u>Presenter</u> : Claudia Benavente, Institute of Microbiology & Infectious Diseases, Faculty of Medicine, University of Calgary, Calgary, Alberta	35
3:20-3:40 PM	<i>“Development of a new immunochromatographic strip assay to detect the infection caused by PRRSV in pigs”</i> <u>Presenter</u> : Maamar Achacha, Arivac Inc., St-Hyacinthe, Québec.	36
3:40 - 4:10 PM	<i>“Johne’s disease in cattle and Crohn’s disease in humans – linked diseases?”</i> <u>Presenter</u> : Herman Barkema, Department of Production Animal Health, University of Calgary – Faculty of Veterinary Medicine (UCVM), Calgary, Alberta.	37
4:35 - 5:00 PM	Bus trip to the Clinical Skills Building (CSB), Spy Hill Campus, University of Calgary – Faculty of Veterinary Medicine (UCVM)	

5:00 - 6:00 PM Tea/Coffee – Networking - CSB tours

6:00 - 8:00 PM *Dinner at the CSB*

8:00 PM *Bus returns to the Foothills Campus and to Best Western Village Park Inn*

Which Quality Program is Best for Your Lab?

Grant Maxie¹, Nadine Ryan¹

Clients expect laboratories to produce reliable and reproducible results, without fail. Laboratories typically ensure the credibility of their results through instituting a formal quality program. The basic tenets of any quality program are to “say what you do, do what you say, prove it, and improve upon it”.

Several options are available, each based on similar standard components, and they include:

- **ISO 9001:2008** (Quality management systems),
- **ISO/IEC 17025** (General requirements for the competence of testing and calibration laboratories),
- **AAVLD** (Requirements for an accredited veterinary medical diagnostic laboratory).

ISO 9001 focuses on **customer satisfaction** and managing the organization’s interconnected process to meet the customer’s and regulatory requirements. It includes management responsibility, resource management, product (service) realization, and measurement, analysis and improvement. The requirements of ISO 9001 are generic and apply to all organizations.

ISO/IEC 17025 and AAVLD expand upon the ISO 9001 generic requirements, rewriting to specific laboratory requirements and adding technical requirements relevant to the laboratory’s competence to produce accurate and reliable results.

Federal labs (CFIA, USDA) typically operate under an ISO/IEC 17025 standard, and work subcontracted from them must meet this standard. Biennial external audits are required, and accreditation is scope-specific (limited to audited tests) and posted on the website of the national accrediting body, e.g., Standards Council of Canada (SCC).

ISO 9001 registration involves annual surveillance audits and a registration audit every 3 years and. It is general in scope, and does not involve assessment of competence to perform individual tests.

AAVLD accreditation has evolved from a peer-help process to now be based on the OIE Quality Standard and Guidelines for Veterinary Laboratories: Infectious Diseases, 2008, which is consistent with the ISO/IEC 17025 standard. External audits vary, with a maximum of 5 years between audits.

The bottom line for any of the paths chosen is that clients are assured of quality, and the laboratory benefits from continuous improvement.

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

Rapid Identification of Bovine Mastitis Pathogens by High Resolution Melt Analysis of 16S rDNA Sequences

Praseeda Ajitkumar¹, Herman W. Barkema¹, Jeroen De Buck¹

Two novel, rapid assays were developed for speciation of bacterial mastitis pathogens using high-resolution melt analysis (HRMA) of 16S rDNA sequences. Independent DNA extractions were carried out on duplicate cultures of 12 major udder pathogen species and 13 coagulase-negative staphylococci (CNS). Real-time PCR amplification of 16S rRNA gene fragment, spanning the variable region V5 and V6 was performed with a resulting amplicon of 290bp. For the CNS species, a 16S rRNA gene fragment including the variable region V1 and V2 was selected with a resulting amplicon of 215bp. Melt curves were generated, analysed and compared with the genetic differences in the respective target sequences of the different bacterial species. Of the 12 major pathogens, 10 had distinct melt curves. Complete discrimination was achieved in an additional step by creating heteroduplexes by adding known template to the reaction mix for the few overlapping species. All CNS species were reproducibly discriminated based on their distinct melt curves. The present study revealed that broad-range real-time PCR with HRMA can be used as a powerful, fast, low-cost tool for the differentiation of clinically important bacterial mastitis pathogens.

¹Department of Production Animal Health, Faculty of Veterinary Medicine, University of Calgary, Calgary, Alberta

Equine Inflammatory Airway Disease (IAD): Epidemiology, Diagnosis and Treatment of This Common Th2 Type Inflammatory Disease

Renaud Leguillette¹

Although equine Inflammatory Airway Disease (IAD) has been recently defined in a consensus publication, little is known on the epidemiology, pathophysiology and treatment of the disease. We performed a large field study showing that mild lower airway inflammation compatible with IAD has as high of a prevalence as 52% in the horse population of Alberta. Respiratory clinical signs during exercise affected 41% of the horses with mild lower airway inflammation. The diagnosis of IAD is based on history, respiratory clinical signs, bronchoalveolar lavage cytology and eventually lung bronchoprovocation challenges. Little is known on the pathophysiology of IAD. We characterized the cytokines expression profile in the BAL fluid of horses affected with IAD. We found that it is compatible with a Th2 type inflammatory reaction and that IL-17 and chemotactic cytokines are important in the pathophysiology of IAD. Because no data is available on the treatment of IAD, it is commonly treated with the empirical use of steroids. We conducted clinical trials showing that both systemic and inhaled steroids are effective at decreasing the airway hypersensitivity observed during bronchoprovocation challenges in IAD horses. Steroids are however not effective at decreasing the accumulation of inflammatory cells in the BAL fluid. This may be explained by persistent expression levels of chemotactic cytokines in the BAL fluid of IAD horses treated with steroids. Further studies are necessary to better characterize the sub-phenotypes included in the definition of IAD and the pathophysiology of the disease.

¹ Assistant Professor, Department of Veterinary Clinical and Diagnostic Sciences, Faculty of Veterinary Medicine, University of Calgary

Morphologic and Molecular Pathogenesis Study of Lesions in Condemned Porcine Kidneys

Claudia Benavente¹, Glen Armstrong¹, Rebekah De Vinney¹, Carmen Fuentealba^{1,2}, Catherine Anne Muckle³

Kidney lesions are an important cause of tissue condemnation in slaughterhouses. In addition to the potential public health implications, organ condemnations have a significant economic impact on the food animal industry. Condemned kidneys of pigs from the Calgary area (market hogs) were examined by bacterial culture, histochemistry and specific molecular tests in order to identify the causative agents prevalent in the region.

Forty kidneys (30 condemned under the general category of “multifocal white spots” and 10 grossly normal) were collected at a slaughter house near Calgary, Alberta and sampled for microbiologic and histological analysis. Macroscopic abnormalities were classified using a 0-3 grade scale, based in the size and distribution of the lesions. Microscopic examination of the lesions included routine histology (H&E) and histochemistry. Bacteriologic examination was performed at the Diagnostic Services Unit, University of Prince Edward Island. Matrix-assisted laser desorption/ ionization (MALDI) time of flight (TOF) mass spectrometry was used for bacteria identification and specific biochemical tests.

Three out of ten control samples (30%) and 21/ 30 (70%) of condemned kidney were positive to pathogenic bacteria in culture media. Gross and histologic changes were associated to detection of *Streptococcus sp.*, *Lactococcus garviae*, *Staphylococcus saprophyticus*, *Sphinobacterum multivorum*, *Acinetobacter spp.*, and *Stenotrophomonas maltophilia*.

Conclusions: Several of the bacteria detected by mass-spectrometry have been described in nosocomial infections in immune-suppressed human patients and may develop multidrug resistance. Western blot analysis in serum samples will be performed to determine if the isolated bacteria caused disease in these pigs.

Microbiology and Infectious Diseases Research Group

¹Faculty of Medicine, University of Calgary

²Faculty of Veterinary Medicine, University of Calgary

³Diagnostic Services Unit, Atlantic Veterinary College, University of Prince Edward Island

Development of a New Immunochromatographic Strip Assay to Detect the Infection Caused by PRRSV in Pigs

Maamar Achacha¹, A. Kheyar¹, A. Bensari¹

A rapid immunochromatographic strip assay (ICSA) was developed for the detection of antibodies specific to nucleocapsid (N) protein of porcine reproductive and respiratory syndrome virus (PRRSV). In this study we have used a new engineered recombinant plasmid (pGEX-N) and a modified E.coli strain to rise the expression cassette to optimize the amino acid sequence at the GST–N junction and to introduce E.coli preferred codons in the recombinant GST-N sequence. Using this new engineered plasmid we succeeded in obtaining highly level expression of soluble PRRS N protein and used it in ICSA as capture antigen. The performance of this assay was evaluated with sera samples from both clinical and experimentally infected piglets. Detection by ICSA was compared with detection by standard, available commercially; indirect enzyme-linked immunosorbent and western blot assays. The ICSA detected antibodies in sera known to contain antibodies to PRRSV in 96.74% (sensitivity) of samples from experimentally infected pigs, the specificity was 99.34% for clinical and experimental serum samples, respectively. These tests were found more simple and easy to use for a rapid and effective diagnosis of PRRS viral infection and could be used as an alternative to the ELISA for the screening of pig infected herds under field conditions.

¹Arivac Inc, St-Hyacinthe, Québec, Canada

Crohn's Disease in Humans and Johne's Disease in Cattle – Linked Diseases?

Herman Barkema¹

The suggestion that *Mycobacterium avium* subspecies *paratuberculosis* (MAP) plays a role in Crohn's disease (CD) is nearly 100 years old. MAP may play a role in the pathogenesis of CD; however, this relationship is not proven. The pathogenesis of CD is likely multifactorial in light of different susceptibility genes and phenotypes of CD. Consequently, MAP may only influence a subset of CD patients. Transmission of MAP to people through milk and meat is possible, but the impact of water is not well studied and may play a larger role than infection by means of animal products. While a final consensus has not been reached regarding the association of MAP with CD, the evidence to support this association is increasing. Future studies are needed to determine if MAP is either: an innocent bystander; an infectious cause in a subset of CD; or whether MAP influences dysregulation of immune response through gene-MAP interactions. Understanding the exact nature of MAP's involvement in human disease is important because of MAP's potential consequences on public health. Finally, irrespective of MAP's relationship with CD, MAP causes JD, which is a serious threat to the cattle industry. Consequently, studying MAP in CD and controlling the outbreak of JD should be a top priority.

¹Department of Production Animal Health, Faculty of Veterinary Medicine, University of Calgary, Calgary, Alberta, Canada

CAHLN / RCTLSA 9th Annual Meeting

Wednesday, June 9, 2010

9:00 AM – 12:00 PM

Theatre 1, Health Sciences Center

Moderator: TBA

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8:00 - 9:00 AM	Morning Tea/ Coffee - Networking	
9:00 - 9:20 AM	<i>"Use of bacterial antimicrobial resistance gene microarray for the identification of Staphylococcus aureus resistance"</i> <u>Presenter:</u> Philippe Garneau, Centre de Recherché en Infectiologie Porcine (CRIP), Faculté de Médecine Vétérinaire, Université de Montréal, Saint-Hyacinthe, Québec	39
9:20 - 9:40 AM	<i>"A scheme for comprehensive typing of Mycobacterium avium subspecies paratuberculosis strains using real-time high resolution melting"</i> <u>Presenter:</u> Joel David, Department of Production Animal Health, University of Calgary – Faculty of Veterinary Medicine (UCVM), Calgary, Alberta	40
9:40 - 10:00 AM	<i>"Detection of bovine lymphotropic herpesvirus DNA in tissues of a bovine aborted fetus in Québec"</i> <u>Presenter:</u> Carl A. Gagnon, Service de Diagnostic, Centre de Recherché en Infectiologie Porcine (CRIP), Groupe de Recherche sur les Maladies Infectieuses du Porc (GREMIP), Faculté de Médecine Vétérinaire, Université de Montréal, Saint-Hyacinthe, Québec	41
10:00 - 10:30 AM	Coffee Break-Networking	
10:30 - 11:00 AM	<i>"Overview of Diagnostic Services at UCVM – challenges and future directions"</i> <u>Presenter:</u> Oscar Illanes, Department of Production Animal Health, University of Calgary – Faculty of Veterinary Medicine (UCVM), Calgary, Alberta	
11:00 - 11:20 AM	<i>"Progressive juvenile-onset myeloencephalopathy in Angus cattle"</i> <u>Presenter:</u> Oscar Illanes, Department of Production Animal Health, University of Calgary – Faculty of Veterinary Medicine (UCVM), Calgary, Alberta	42
11:20 - 11:40 AM	<i>Presentation of the Graduate Student Award and Laboratorian of the Year Award - CAHLN/ RCTLSA National Steering Committee</i>	
11:40 - 11:50 AM	<i>Closing Remarks</i>	
12:00 –1:00 PM	<i>Lunch</i>	

Use of a Bacterial Antimicrobial Resistance Gene Microarray for the Identification of *Staphylococcus Aureus* Resistance

Philippe Garneau¹, Serge Messier¹, Marie Archambault¹, Josée Harel^{1,2}, Donald Tremblay², Christine Maynard³, Luc Masson³, Olivia Labrecque⁴

As diagnostic and surveillance activities are vital to determining measures needed to control antimicrobial resistance (AMR), new and rapid laboratory methods are necessary to facilitate this important effort. DNA microarray technology allows the detection of a large number of genes in a single reaction. This technology is simple, specific and high-throughput. We have developed a bacterial AMR gene DNA microarray that will allow rapid AMR gene screening for all Gram positive and Gram negative bacteria. A prototype microarray was designed using a 70-mer based oligonucleotide set targeting AMR genes of Gram-negative and Gram-positive bacteria. In the present version, the microarray consists of 182 oligonucleotides corresponding to 166 different acquired AMR gene targets, covering most of the resistance genes found in both Gram-negative and -positive bacteria. A test study was performed on a collection of *Staphylococcus aureus* isolates from milk samples from dairy farms in Québec, Canada. The reproducibility of the hybridizations was determined, and the microarray results were compared with those obtained by phenotypic resistance tests (either MIC or Kirby-Bauer). The bacterial antimicrobial resistance gene DNA microarray provides detailed relevant information on *S. aureus* isolates by detecting the presence or absence of a large number of AMR genes simultaneously in a single assay. The hybridizations showed that the 38 AMR resistant *S. aureus* isolates possessed at least one AMR gene.

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A Scheme for Comprehensive Typing of *Mycobacterium Avium* Subspecies *Paratuberculosis* Strains Using Real-time High Resolution Melting

Joel David¹, Jeroen De Buck¹, Elena Castellanos²

Johne's disease is chronic granulomatous enteritis in ruminants caused by *Mycobacterium avium* subspecies *paratuberculosis* (MAP). Genotyping of isolates contributes to the understanding of their molecular epidemiology, host range and different clinical presentations. Currently, techniques to detect and type these strains are laborious and expensive. High resolution melting analysis (HRM) is a quick single tube technique to differentiate genetic variation as small as SNPs (single nucleotide polymorphism). We developed a series of HRM based assays to type *Mycobacterium avium* complex strains with increasing resolution. First, two individual HRM assays were developed based on the nucleotide polymorphisms in the PPE genes to first differentiate MAP from other members of the *Mycobacterium avium* complex and second differentiate between the three subtypes of MAP. A third HRM assay was developed based on the short sequence repeats (SSR) variations found in MAP strains to further differentiate the MAP clinical isolates at a higher discrimination. Three SSR loci (G1, G2, GGT) with a high discriminatory index were chosen for this purpose. The alleles of G1 and G2 could be discriminated directly by HRM. Resolution of the GGT allele required heteroduplex formation by adding a known template. Fifty isolates obtained from 12 Alberta herds with low MAP prevalence were analyzed by the SSR HRM technique leading to a better understanding of the distribution of different strain types among Central and Southern Alberta dairy farms. The combined set of HRM based genotyping assays will allow a comprehensive insight in the diversity of *Map* in dairy herds on a large scale. It has the potential to provide a clear view of the spread of this organism between farms. It will also allow identification of the sources of contamination and it will help determine if the same *Map* subtypes are shared with wildlife, the environment and potentially humans.

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Detection of bovine lymphotropic herpesvirus DNA in tissues of a bovine aborted fetus in Québec

Carl A. Gagnon^{1,2,3}, Ossama Allam^{2,3}, Richard Drolet¹, Donald Tremblay¹

In 2008, the Molecular Biology Diagnostic Laboratory (MBDL) of the Faculty of veterinary medicine of the University of Montreal started a research project to investigate the etiological agents involved in cow abortion in Québec. Several RT-PCR and PCR based assays were done on placenta and tissues of aborted fetuses that were submitted to the MBDL for the detection of multiple bovine pathogens such as bovine viral diarrhea virus (BVDV), infectious bovine rhinotracheitis virus (IBR), *Neospora caninum*, *Leptospira* spp. and *Coxiella burnetii*. From the 26 submitted cases of bovine abortion that were tested, none were positive for BVDV and *Leptospira* spp., whereas 3.8%, 11.5% and 3.8% were positive for IBR, *Neospora caninum*, and *Coxiella burnetii*, respectively. In addition to the usual PCR assays, a pan-herpesvirus nested-PCR assay which is able to detect a broad spectrum of herpesvirus species was conducted on the samples. In regards to this pan-herpesvirus nested-PCR assay, excluding the IBR positive cases, one submitted sample (FMV09-1125585) gave a positive reaction on the DNA extracted from the placenta. PCR fragment sequencing and GenBank comparison by BLAST showed high homology with the DPOL viral gene of a bovine lymphotropic herpesvirus (BLHV), which is a virus classified in the *Herpesviridae* family within the subfamily *Gammaherpesvirinae*. To our knowledge, it is the first time that BLHV is found in the bovine species in Canada. In the present case it is very difficult to ascertain that BLHV has a role to play in the cause of the abortion since the presence of *Neospora caninum* was confirmed and no histopathological lesion suggestive of a viral infection could be found in the BLHV nested-PCR positive tissues. Nonetheless, it is important to report the presence of BLHV in Canada because it is the first step toward the country improvement of bovine pathogens surveillance.

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Progressive Juvenile-onset Myeloencephalopathy in Angus Cattle

Oscar Illanes¹, Michel Levy¹, Eugene Janzen¹

Over a two week period four, approximately 8-months old Angus calves from a cow –calf operation located in Pincher Creek, Alberta, developed neurologic signs characterized by hind limb ataxia and mild forelimb hypermetria. These animals, sired but the same bull, had been kept in the same pasture which contained the remains of an old burned house. Neurologic examination revealed normal mentation and no evidence of cranial nerve deficits or muscle weakness. Clinical findings were consistent with multifocal or diffuse spinal cord disease. Ataxia was progressive leading eventually to recumbency requiring euthanasia within a 2 month period. The owner indicated that in the previous year 3 other calves, descendants from the same Angus bull, also developed similar clinical disease.

Post-mortem examination revealed no significant gross findings. Microscopically, axonal degeneration and dilatation of myelin sheaths was observed diffusely within spinal cord funiculi and caudal brain stem. All segments of the cord were affected. Minimal to mild axonal degeneration was also observed within the sciatic nerves. A large basophilic protozoan cyst filled with abundant zoites was present within the grey matter of the thoracic spinal cord in only one calf. The cyst did not elicit an inflammatory response and no evidence of necrosis or perivascular cuffing, typical of protozoan myelitis, was present in any of the sections examined from this animal. Small isolated foci of inflammation were rarely found in the spinal cord of two other calves but in the absence of protozoa. Toxicologic testing for the detection of lead within blood and tissues was negative. Vitamin E, magnesium, iron, copper, zinc and molybdenum levels in kidney or liver were within normal range. The history, clinical signs and diffuse spinal cord microscopic findings in these calves suggested a possible genetic etiology similar to the progressive spinal myelinopathy reported in Murray Grey cattle or bovine progressive myeloencephalopathy (“weaver syndrome”) in Brown Swiss cattle. The diagnostic implications of the inflammatory lesions and the additional testing needed to rule out or confirm a diagnosis will be discussed.

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