11th Annual meeting of the Canadian Animal Health Laboratorians Network (CAHLN)

June 3-6, 2012

One Health – Emerging Diseases

11e réunion annuelle du Réseau canadien des travailleurs des laboratoires de santé (RCTLSA)

3 au 6 juin, 2012

Une seule santé - maladies émergentes

Hosted by:
Canadian Food Inspection Agency
National Centre for Foreign Animal Disease
and
Manitoba Agriculture Food and Rural Initiative
Winnipeg, Manitoba

11th Annual meeting of the Canadian Animal Health Laboratorians Network (CAHLN) June 3-6, 2012

Canadian Food Inspection Agency National Centre for Foreign Animal Disease and Manitoba Agriculture Food and Rural Initiative Winnipeg, Manitoba

INTRODUCTION

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

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

Previous annual meetings have been held in:
2002 – Ottawa (CFIA OLF)
2003 – Ottawa (CFIA OLF)
2004 – Guelph (Animal Health Laboratory, Ontario Veterinary College)
2005 – St Hyacinthe (Faculté de médicine vétérinaire)
2006 – Ottawa (CFIA OLF)
2007 – Saskatoon (Western College of Veterinary Medicine / Prairie Diagnostic Services)
2008 – Ottawa (CFIA OLF)
2009 – Charlottetown (AVC – UPEI)
2010 – Calgary (UCVM)
2011 – Animal Health Laboratory, Ontario Veterinary College, University of Guelph

The 2012 local organizing committee is comprised of: John Copps (chair), Ann Copps, Deidre Ridd, Greg McLean, and Michele Roy.

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Centre national des maladies animales exotique, (Agence canadienne d'inspections des aliments) et le Manitoba Agriculture Food and Rural Initiative Winnipeg, Manitoba

INTRODUCTION

Le RCTLSA a été crée 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 (anatomique, clinique), en immunologie, en virologie, en parasitologie, en surveillance/épidémiologie, en toxicologie et en biologie moléculaire.

Les Congrès annuel précédents ont eu lieu à :
2002 – Ottawa (CFIA OLF)
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2011 – Animal Health Laboratory, Ontario Veterinary College, University of Guelph

The comité local d'organisation pour le congrès 2011 comprend John Copps (chair), Ann Copps, Deidre Ridd, Greg McLean, et Michele Roy.

CAHLN Laboratorian of the Year

Prix du diagnosticien du RCTLSA

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

Le Réseau canadien des travailleurs des laboratoires de santé animale (RCTLSA) remet à chaque année le prix d'une plaque à un travailleur de laboratoire basé sur ses contributions remarquables à la médecine vétérinaire de laboratoire au Canada. Un candidat pourrait être un diagnosticien exceptionnel, éducateur, chercheur, mentor de futurs diagnosticiens de laboratoire ou autre collaborateur.

Past Recipients / gagnants des années passées :
2003 – Lloyd Spencer, CFIA
2004 – Ian Barker, OVC
2005 – Marcelo Gottschalk, FMV
2006 – John Robinson, MAL
2007 – John Fairbrother, FMV
2008 – W.D.G (Bill) Yates, CFIA
2009 – Gerald R. Johnson, AVC
2010 – Ted Clark, Calgary
2011 – Josepha Patricia DeLay, OVC

CAHLN Graduate Student Presentation Award

RCTLSA Prix de présentation d'un étudiant diplômé

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 à un étudiant diplômé qui a présenté le meilleur exposé basé sur la qualité de la présentation dan 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 à notre base de connaissances, la compréhension de l'étudiant et sa présentation de la matière, ainsi que leur capacité de répondre aux questions.

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

Past Recipients / gagnants des années passées :
2003 – Sherry Andrews, WCVM
2004 – Noel Harrington, OVC
2005 – Guillaume Bruant, FMV
2006 – Yuanmu Fang, WCVM
2007 – Kathi Ellis, WCVM
2008 – Angela Catford, OVC
2009 – Raphael Vanderstichel, AVC
2010 – Guilherme Gomes Verocai, UCVM
2011 – Olivier Cote, OVC

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CAHLN-RCTLSA 2012 and
Special Symposium Preliminary Program

Sunday, June 3, 2012

0800-1630 **CAVP-ACPV Annual Pathology Conference**
Manitoba Veterinary Diagnostic Services, MAFRI
545 University Crescent, University of Manitoba campus

1830-2100 **CAHLN / RCTLSA /CAPV-ACPV Evening Reception**
Theatre – Canadian Science Centre for Human and Animal Health (CSCHAH), 1015 Arlington Street

:

1830-2000 CAHLN meeting registration, reception and tour of the Canadian Science Centre for Human and Animal Health

2000-2100 Guest Speaker: Prof. Soren Alexandersen, Executive Director, National Centres for Animal Disease, CFIA – ***One World and the Life of an International Laboratorian - A Viking's perspective***

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IDEXX

CAHLN-RCTLSA 2012 – Monday, June 4 Program
One Health - Emerging Diseases

Registration Desk: 0730-1500 Hrs

Session Theme: Zoonoses – the Animal/Human Interface
Moderator: John Copps

0815 **Official Opening and Welcome** – Martine Dubuc

0830 **Influence of Zoonotic Disease on Human Health** – Richard Rusk

0900 **Canadian Swine Health Intelligence Network** - Brad Chappell

0930 **Zoonotics in Slaughterhouses** – Yves Robinson

*1000-1030 – **Break, exhibits** – Theatre Lobby and Orientation Room*

1030 **Diagnostic and Surveillance Approaches for Mosquito-Borne Bunyaviruses of Public and Animal Health Importance** – Michael Drebot

1100 **Emerging Pathogens of Veterinary Importance: Schmallenberg Virus –** Soren Alexandersen

1130 **Emergency Preparedness for Emerging Diseases: a Provincial Perspective –** Wayne Lees

1200-1330 ***Lunch, Exhibits** – CSCHAH Cafeteria, Theatre Lobby and Orientation Room*

1230-1330 CAHLN-RCTLSA Business Meeting (open) –Theatre

Session Theme: Networks: Harmonization of Diagnostics and Improvements in Data Delivery
Session moderator: Grant Maxie

1330 **Lessons Learned from Conducting Foreign Animal Disease (FAD) Emergency Exercises in the Veterinary Necropsy Laboratory** – Maria Spinato

1400 **Using Spatial Epidemiological Techniques to Support One Health Investigation of Infectious Diseases –** Chris Green

1430 **Data Sharing Positive Aspects with Regard to Federal-Provincial Movement of Data** – Harold Kloeze

*1500-1530 **Break, exhibits –Theatre Lobby and** Orientation Room*

*1530 **Laboratorian of the Year Presentation***

1530 **Premise ID – Use in Emergency Response** – Heather Martens

1600 **CAHSN Current and Future Opportunities** – Marilyn Jonas

1630 Adjourn

1700-2000 **CAHSN Executive Board meeting** (by invitation) – *Main Boardroom*

The Influence of Zoonotic Disease on Human Health

Richard Rusk

Province of Manitoba
Winnipeg, MB

Infectious diseases have shaped the history of the world for centuries. In recent history there was the thought that these diseases could be eradicated, but to date there have been very few that have. There is continuing evidence that the emergence and re-emergence of zoonotic diseases needs to be considered when planning for future interventions in public health and outbreak management. With the close association that humans have with animals, these diseases will continue to be important for future epidemics and pandemics. Despite the technical and scientific advances that we have, there is still a need for greater collaboration within a One Health agenda. This talk will describe our past and present and then future thoughts for zoonotic disease based on a One Health framework.

Canadian Swine Health Intelligence Network

Brad Chappell

Swine Health Professionals Inc.
Steinbach, MB

J Berezowski[1], C Byra[2], E Brockhoff[3], D Hurnik[4], C Klopfenstein[5], H Kloeze[6]

[1]Ross University School of Veterinary Medicine, St.Kitts, West Indies, [2]Greenbelt Swine Veterinary Services, Chilliwack, BC, Canada, [3]Prairie Swine Health Services, Red Deer, AB, Canada, [4]Atlantic Veterinary College, University of Prince Edward Island, Charlottetown PEI, Canada, [5]Le Centre de développement du porc du Québec, Québec, PQ, Canada, [6]Canadian Food Inspection Agency, Owen Sound, ON, Canada

National animal health surveillance is almost universally aimed at dealing with diseases that impact trade and public health, and some diseases that are of great enough importance to affect the whole industry[1]. The norm among trading nations is for animal health surveillance to be funded by governments, to provide information for government decision makers to make decisions and develop policy on behalf of livestock industries[1]. These programs are, undeniably, of great benefit to livestock industries as a whole, however, they seldom aim to support private veterinarians and their clients in their day to day battles against emerging and ever present endemic diseases that affect productivity and profitability.
The purpose of this project is to design and implement a new swine surveillance network for the Canadian industry. The project will use current information technologies to create real-time swine health intelligence. The goal of the network is to generate intelligence that helps practicing veterinarians and their clients to reduce the impact of disease on Canadian swine farms. The network has been named the Canadian Swine Health Intelligence Network (CSHIN).

The Canadian Swine Health Board (CSHB)[2], an organization aimed at providing leadership, coordination and support in the management of the health of the Canadian swine herd, is leading the implementation of the CSHIN. During 2010, a national project was initiated by the CSHB to evaluate the current state of swine health surveillance across Canada, identify gaps and suggest broad solutions. This was followed by a call for proposals to design the CSHIN and develop a plan for implementation. CSHIN will be created using a series of individual projects, under the umbrella of an overarching, and coordinating project, which will result in the CSHIN becoming operational across Canada in 2012-2013.

In Canada, surveillance for swine diseases is divided between national and provincial governments, with some additional surveillance being conducted as research projects by academic institutions. With the exception of the réseau d'alerte et d'information zoosanitaire (RAIZO)[3] system in Quebec; swine health surveillance is targeted against diseases that impact trade and public health.
Critical success factors in the design of the national surveillance system identified included: creating value for stakeholders and providers of health information by rapidly communicating useful information back to stakeholders, maintaining producer confidentiality, maintaining strict control over public communications and building on existing health infrastructure. Infrastructure that was identified included a web-based veterinary practice management system (Swine Database Services[4]), national surveillance infrastructure (Canadian Animal Health Surveillance Network[5]) and veterinary social networks (RAIZO and the Ontario Swine Health Advisory Board[6]).

The design of the CSHIN is unique in several ways. At its core it is a national surveillance system aimed at providing surveillance intelligence to support practicing swine veterinarians. It includes both a data network and a parallel social network. The data network will include data from a real-time web-based veterinary practice surveillance network. It will feed swine health intelligence into the social network (composed of swine veterinarians, producers, diagnosticians and other swine health experts) that will validate the data, develop and implement strategies to deal with emerging diseases and communicate information as needed to support the strategy taken.

Zoonoses Affecting Slaughterhouse Workers

Yves Robinson

Saint-Hyacinthe Laboratory, Canadian Food Inspection Agency
Saint-Hyacinthe, QC

The red meat processing industry was one of Canada's largest industry with 37,377 employees in1996[1]. In 2006, Canada's read meat processing industry of beef, veal, pork, lamb and horse was employing around 46,000 people[2]. In 2009, the number of poultry establishments in Canada was 191 with 16,851 production workers[3].

Now with over 645 million chickens, turkeys and ducks, and 21 million hogs, 3.5 million cattle and calves, and alternative livestock (deer, elk, bison, wild boar and rabbits) slaughtered every year in Canada [4], slaughterhouse workers are known as high risk group for zoonoses.

With such large number of animals brought for slaughter and the close contact with animal products and tissues such as blood, viscera, fetal and placental tissues, urine, and hide contaminated with feces there is a high probability that zoonotic diseases are transmitted orally or through the skin, even those with low prevalence.

The working environment which is frequently contaminated, dusty and humid can also be an important source of pathogenic microorganisms that can infect those workers through inhalation.

Rabies, tuberculosis, brucellosis, Q fever, chlamydiosis, anthrax, erysipeloid, steptocoocsis (*Streptococcus suis*), poxviruses, influenza, salmonellosis, campylobacteriosis, *Escherichia coli* infections have been reported as professional zoonoses of slaughterhouse workers.

Many animals serve as reservoirs for zoonotic infections and are sent for slaughter with no evidence of disease. On the other hand, some animals show evidence of disease when brought for slaughter.

The purpose of this presentation is to illustrate some potential zoonotic conditions seen in Canadian livestock sent for slaughter.

[1] Statistics Canada Catalogue 32-250
[2] Canadian Agri-food Trade Alliance, Canada's Meat Processing Sector www.cafta.org
[3], Statistics Canada, Canadian Industry Statistics, Annual Survey of Manufactures, Poultry processing (NAICS 311615)
[4] Agriculture and Agri-Food Canada. www.agr.ca

Diagnostic and Surveillance Approaches for Mosquito-Borne Bunyaviruses of Public and Animal Health Importance

Michael A. Drebot

National Microbiology Laboratory, Public Health Agency of Canada
Winnipeg, MB

California serogroup (CSG) and Cache Valley viruses (CVV) are mosquito-borne pathogens (arboviruses) belonging to the genus *Bunyavirus* (Family Bunyaviridae) present throughout North America. These bunyaviruses circulate in specific transmission cycles consisting of a number of vertebrate hosts and various mosquito vectors, including several species of *Aedes* and other non-Culex mosquitoes. The two most common CSG viruses found in Canada are Jamestown Canyon (JC) and snowshoe hare (SSH) viruses. Although the majority of human exposures to these viruses result in mild disease, both CSG and CV viruses can cause febrile and neurological illnesses similar to that observed with West Nile virus (WNV).

Both SSH and JC viruses have also been associated with diseases in horses and found to infect a variety of other livestock including sheep and cattle. SSH virus was previously identified to be the cause of equine encephalitis in horses from Ontario and Saskatchewan during the mid to late 80's. JC virus has been isolated from vesicular lesions in the tongue and lower lip of horses with accompanying symptoms of emaciation, weakness and anorexia. CVV can cause fetal malformations by intrauterine inoculation of the virus in pregnant sheep and has been associated with outbreaks resulting in lamb deformities and embryonic death.

Recently, a variety of diagnostic procedures for identifying exposures to these agents have been developed at the National Microbiology Laboratory in Winnipeg. Both molecular (PCR) and serological based assays are available to identify CSG and CVV infections in both livestock and humans. In the past number of years JC and SSH viruses have been implicated in confirmed human cases of meningitis and encephalitis throughout Canada, however, the exact burden of disease remains to be determined. Serosurveys have shown that horses and other livestock from various provinces display a significant degree of CSG seroprevalence ranging from 25-50%. CSG-associated neurological disease in Canadian horses may occur at higher rates than previously documented. Human exposures to CVV have also been documented in various prairie provinces in 2009 and 2010. A CVV genetic variant has recently been isolated from deformed lambs in Ontario flocks and this finding indicates that this bunyavirus may be causing sporadic outbreaks throughout the country. Increased awareness of these agents as potential pathogens impacting on public and animal heath is warranted.

Emerging Pathogens of Veterinary Importance: Schmallenberg virus

Soren Alexandersen and Zhidong Zhang

National Centres for Animal Disease, Canadian Food Inspection Agency
Winnipeg, MB

New and emerging pathogens are becoming more important and need to be looked at in the context of the changing interactions between animals, the environment, and humans; the so-called “One World – One Health” concept. Changes include increased movement of animals, products and people, and climate and other environmental changes caused by human activities and affecting wildlife populations and their interactions with livestock, pets and humans. Around 600 pathogens are known in livestock and the highest risk of new and emerging infections, in both animals and humans, are from RNA and single stranded (SS) DNA viruses as their replication involves polymerases with poor proof-reading ability causing rapid genome variability (may be further enhanced by recombination and/or reassortment) which necessitates a limited genome size which, in turn, facilitate rapid and high level replication. Consequently, such viruses consist of a swarm of slightly different genomes, by some called quasispecies, which may, given the right opportunity, enable infection of a different species or expansion into a previously naive environment.

An apparently new virus, the so-called Schmallenberg virus (SBV), has been detected in ruminants (cattle, sheep, goats and bison) in Germany and Netherlands followed by Belgium, UK, France, Luxembourg, Italy and Spain. The virus has now (April 2012) been detected in more than 3000 farms with spread likely facilitated by biting midges (Culicoides) or mosquitoes during the Summer and Fall of 2011. In the initial stage, i.e. from August 2011, clinical signs were mild, only seen in cattle and transient (fever, drop in milk production and in some cases diarrhoea) while significant effects were detected later, basically from December 2011, in the form of miscarriages and stillbirths associated with congenital malformations in ruminants.

The virus was first detected in November 2011 by metagenomic analysis and virus isolation from samples collected near the German Town of Schmallenberg. The virus belongs to the Simbu serogroup in the *Orthobunyavirus* genus of the *Bunyaviridae* Family of viruses. All known Orthobunyaviruses are vector-borne, mainly transmitted by biting midges and mosquitoes. The Simbu serogroup alone contains around 25 different viruses and many are found in ruminant livestock and wildlife. None of the Simbu serogroup viruses have ever been detected before in Western Europe, however some of them are relatively common in Asia, Australia, Africa and the Americas. Only a few of these viruses cause clinical disease, however, several of them may cause significant damage to the developing ruminant foetus. The origin of SBV is currently unknown; however, it may have been brought to Western Europe by infected midges, similarly to the situation for bluetongue virus serotype 8 a few years back.

Although two members of the Simbu serogroup (Oropouche and Iquitos virus), cause illness in humans in Brazil and Peru, SBV genome data indicate that it is not closely related to these viruses, but closer related to Akabane virus, causing similar disease in ruminants in Japan and Australia, and to Shamonda virus detected in cattle in Africa and Japan. Both of these viruses do not cause human disease and there is no evidence that SBV can cause illness in humans although it cannot be formally excluded at this stage.

Bunyaviruses are segmented, single-stranded (primarily negative sense) RNA viruses with 3 genome segments, designated S, M and L. The full sequence of SBV has been deposited in public databases. Diagnostic capacity includes two real-time RT-PCRs for the S and the L gene developed by the German National Laboratory. The S gene assay appears to be the most sensitive, but both assays need further validation. NCFAD has also set up two conventional RT-PCRs for partial sequencing and have made artificial RNA controls for the real time RT-PCRs. The virus has been isolated in cell culture at the German Laboratory, grows well on BHK-cells and shown to cause mild disease (fever, and diarrhoea) and a short vireamia in inoculated cattle. For serology, virus/serum neutralisation test (VNT/SNT) and an immuno-fluorescence assay have been established and ELISAs are under development; all needing further validation. NCFAD has set up VNT/SNT and an immuno-peroxidase assay (IPA). Limited testing at NCFAD of samples associated with abortions/malformations in sheep/lambs has been negative for SBV and for antibodies to SBV, however, testing by the National Microbiology Laboratory indicated presence of antibodies to the Cache Valley virus, an *Orthobunyavirus* in the Bunyamwera serogroup which may cause congenital malformations in sheep and possibly also disease in humans and known to be present in North America.

Although none of the infections caused by the viruses of the Simbu serogroup are notifiable to OIE, several countries have reported to the OIE based on this being an emerging infection and some countries in the EU are considering making/or have already made it nationally notifiable. Some countries have responded with trade restrictions, e.g. Russia and the USA which have placed restrictions on semen and embryos from affected areas. Also Canada is considering mitigation measures concerning imports of semen/embryos, as a possibility of transmission by artificial insemination or embryo transfer can not be excluded. At present, no vaccine is available.

Preparing for animal emergencies: a provincial perspective

V. Wayne Lees

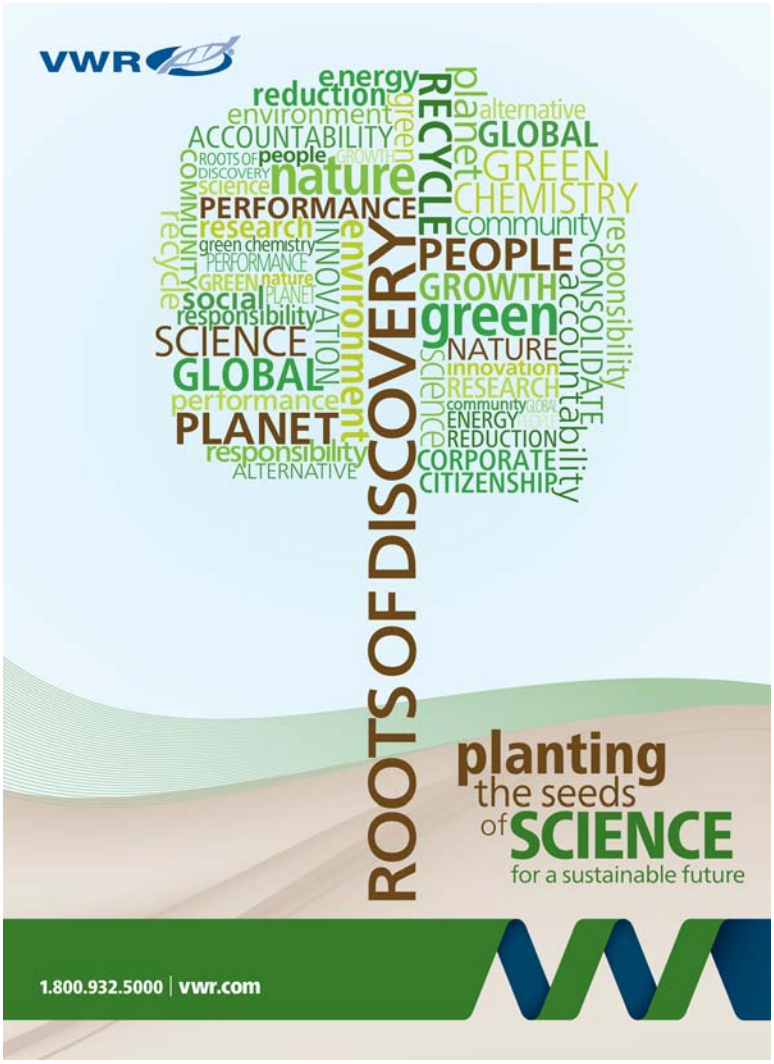
Manitoba Agriculture, Food and Rural Initiatives
Winnipeg, MB

Provinces and territories deal with a wide variety of emergencies that have an animal component. Emerging and zoonotic diseases fall largely within provincial jurisdiction, as do animal welfare issues, and physical threats, such as from flood or fire. This requires adopting an all hazards approach that is nimble, flexible and collaborative. It is important to develop the plans and relationships well in advance of any emergency, so that all players know who will make the decisions and what role each organization will play. This is especially critical for dealing with the first 24-72 hours, when the situation is still evolving and information is often sketchy. The ForeCAN model provides a useful checklist for determining if we have covered all of the components within each phase of emergency management (prevention, preparation, response, recovery). These essential components are:

- Organization and decision making
- Science and technology
- Expertise and personnel
- Policy, law and regulation
- Information and data sharing

Too often, we focus only on one or two of these areas without considering the whole emergency response system. One initiative to address some of these components was the Canadian Animal Health Surveillance Network (CAHSN). CAHSN was originally conceived to be a national laboratory network, surveillance/intelligence network, and platform for sharing data among animal and human health organizations.

As we move forward to prepare for emerging threats, there are needed some structural improvements to Canada's federal and provincial/territorial animal emergency capacity. A clear understanding of roles and responsibilities (governance) must be developed among all stakeholders. Partnerships must be created to bring all of the affected parties together to create solutions. (One example might be a mechanism to facilitate provinces and territories to share expertise during an emergency.) We have a National Farmed Animal Health and Welfare Strategy, but we need to explore ways to operationalize it. This will require leadership, vision and innovative thinking.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Lessons learned from conducting foreign animal disease (FAD) emergency exercises in the veterinary necropsy laboratory

Maria Spinato[1], Jan Shapiro[2]

Animal Health Laboratory, Laboratory Services Division, University of Guelph, Guelph[1], ON, Kemptville[2], ON

Emergency planning has become an important strategic feature of veterinary diagnostic laboratory operations. In September, 2011, the Animal Health Laboratory, University of Guelph conducted full-scale foreign animal disease (FAD) simulations at both the Guelph and Kemptville necropsies laboratories. These were held in conjunction with a broader provincial FAD exercise testing the interactions among Canadian Food Inspection Agency and various swine industry organizations in response to a suspected outbreak of 'standard swine fever'.

The goals of this exercise were:

1) To test and validate the standard operating procedures regarding management of a FAD investigation in the necropsy laboratory, and;
2) To evaluate joint co-ordination and communication among the AHL, University of Guelph, and the CFIA.

Independent evaluators at both locations were provided with an evaluation guide that outlined specific objectives, expected actions and desired timelines. Additional qualitative evaluations were elicited from AHL and CFIA staff participating in the exercise. Evaluators generated a detailed summary of their observations and recommendations, which included:

1) Participants were well-prepared, and the specific objectives of evaluating the FAD SOPs were fully met. All tasks were completed within the suggested time limits.
2) Decontamination and disinfection procedures need to be revised and expanded to address CFIA concerns regarding adequate biocontainment.
3) Minor facilities modifications and additional physical resources were identified.

The FAD exercise was deemed a success at both AHL necropsy laboratories. Plans are underway to address the recommendations of evaluators. **Full-scale operational exercises are critical to maintaining emergency preparedness status, as they provide staff with an opportunity to practice procedures in real time, and facilitate gap analysis.** Moreover, these simulations offer opportunities to interact with local stakeholders and animal health partners, thereby improving co-ordination during an actual emergency. It is anticipated that the AHL will conduct annual table-top or operational exercises to enhance and maintain its emergency preparedness status.

Using Spatial Epidemiological Techniques to support One Health Investigation of Infectious Diseases[1]

Chris Green

[1]Chief Veterinary Office, Ministry of Agriculture, Food and Rural Initiatives
Winnipeg, Manitoba

Increasingly, it is acknowledged that the health of people, animals and our environment are inextricably inter-connected in complex ways. The health and sustainability challenges associated with rapid globalization, urbanization, climate change, intensification of food production systems, and environmental degradation are unprecedented in human history. To protect humans from the impact of emerging infectious diseases in this unpredictable and rapidly emerging context, it is critical that we develop the scientific and investigative approaches to understand how human, animal and environmental health are dynamically connected. However, the scientific methods to empirically investigate these relationships from a One Health perspective are challenging and often expensive to implement. They require assembling data from diverse domains and connecting them together in a ways that are policy and program relevant.

This presentation will discuss one method of assembling and "gluing" together data from across the human, animal and environmental continuums using spatial epidemiological techniques. An overview of relevant spatio-epidemiological methods will be described along with a number of case examples illustrating how they have been used to investigate West Nile Virus, Campylobacter and Salmonella enteriditis infection in Manitoba. The presentation will also describe an analytical framework for spatial investigation across the human, animal and environmental continuums that we are building to partially automate this work.

Data Sharing: Positive Aspects with Regard to Federal-Provincial Movement of Data

Harold Kloeze

Animal Health Science Division, Canadian Food Inspection Agency
Owen Sound, ON

All countries have unique characteristics and this is no less true in Canada than other countries. While those who work in the field of animal health diagnostics and disease control may take our issues for granted they do present problems for disease control and coordinated diagnostics. We have a relatively small population spread over 5000 km; we have fractured legislative authorities between the federal government and provinces with respect to animal diseases which is only complicated further in the case of zoonotic diseases by adding in health agencies; we have various types of laboratories dealing with animal health in Canada including CFIA laboratories, provincial laboratories, academic laboratories, laboratories controlled by partnerships and private laboratories; and finally we have federal and provincial laws regarding privacy and authority over veterinarians is provincially based and not uniform across Canada.

In order to effectively manage animal diseases nationally, for example a severe outbreak of a reportable or zoonotic disease or for national surveillance, it is increasingly necessary for the various agencies to act in a coordinated fashion as no one group has the financial or human resources to do this in isolation. This predicates that cooperation and data sharing will be needed.

Obvious benefits of data sharing include the ability to get a national picture of disease occurrence and prevalence, the ability to report internationally and the ability to respond in a coordinated fashion, and faster detection of emerging and other important diseases. Less obvious benefits are that data sharing allows a platform for evaluation of legal and privacy risks, the solution of technical issues with the IT infrastructure needed, the development of reporting and analysis tools and the provision of intelligence so that informed decisions can be made. Perhaps the most important benefit is the development of trust and confidence in the participants with regards to prevention of potential negative impacts of information sharing. This trust is integral to well functioning partnerships and allows further sharing when necessary.

The Manitoba Premises Identification Program – Minimizing the Impact of Animal Health Emergencies

Heather Martens and David Hunt

Manitoba Agriculture, Food and Rural Initiatives
Winnipeg, MB

In September 2010, the Province of Manitoba implemented a mandatory Premises Identification Program as part of the National Agriculture and Food Traceability Strategy and to serve as the foundation for the newly developed provincial emergency management decision support system. Since then, the information collected by this program has been utilized to prevent, prepare, respond and recover from more than 16 animal health emergencies including disease investigations and natural disaster responses to wildfire and spring flood events. This presentation will describe the Premises Identification Program and provide an overview of how it has been used to minimize the impact of animal health emergencies on individuals, communities and livestock sectors.

CAHSN – Challenges and Opportunities

Marilyn Jonas

Prairie Diagnostic Services
Saskatoon, SK

The Canadian Animal Health Surveillance Network (CAHSN) is a network of publicly funded federal, provincial and university animal health laboratories. The network, which is emulated off a similar structure in the U.S, was founded in 2005 with support from a CRTI grant. The objective of network is two fold: to provide a national early warning system for serious animal disease threats that may impact animal and public health; and to provide expertise and surge capacity to support testing and diagnosis of serious animal disease impacting animals in Canada.

Seven years after its formation and two years after the end of the CRTI grant, the laboratory network has set up a governance structure and regular communication forums. It has a data base infrastructure in place and has initiated work on data sharing agreements. Enhanced bio-safety laboratories, trained staff and testing for selected foreign animal disease is in place in a number of provincial jurisdictions. Most of the laboratories either have ISO/IEC 17025 certification or are moving towards this standard.

Challenges involve lack of sufficient resources to maintain current testing capacity and new development, the need for additional resources and focus to begin data collection, compilation and analysis, and managing diverse capacity and interests across the network. Opportunities involve building on the infrastructure that has been put in place to date and renewing leadership, strategic focus and funding. Experience gained over the last several years along with a growing interest in disease surveillance and management among provincial jurisdictions and industry groups will help determine options and inform decisions on the way forward.

CAHLN-RCTLSA 2012 – Tuesday, June 5 Program
One Health – Emerging Diseases

Registration Desk: 0730-1500 Hrs

Session Theme: Exotic Animal Disease Threats to Animals and Man
Moderator: Mark Swendrowski

0830 **Emerging Vector Borne Disease Threats -** Robbin Lindsay

0900 **Diagnostic Developments for High Consequence Animal Pathogens –** Hana Weingartl

0930 **Diagnostic Developments for High Consequence Human Pathogens -** Gary Kobinger

1000-1030 - ***Break, exhibits –*** *Theatre Lobby and Orientation Room*

1030 **International Twinning of Reference Labs and Harmonization of Diagnostics -** John Pasick

1100 **Sheep and Goat Pox Viral Diagnostics and Vaccine Development Collaboration** - Shawn Babiuk

1120 **Development of Vaccines for the Prevention of Viral Diseases in Livestock in Africa -** Hani Boshra

1140 **Evaluation of Cell Substrates and the Development of RT-PCR and Real-Time RT-PCR Assays for Peste des Petits Ruminants** - Thang Truong

1200-1300 - ***Lunch, exhibits*** *– CSCHAH cafeteria, Theatre Lobby and Orientation Room*

Session Moderator: Hana Weingartl

1300 **Nipah Virus Infects Specific Subsets of Porcine Peripheral Blood Mononuclear Cells -** Beata Stachowiak

1320 **A New Novel Penside Strip Test for the Detection of Nipah Virus in Swine** - Mingyi Li

1340 **Immune Parameters Correlating with Protection against Ebola Virus Infection in Rodents and Nonhuman Primates -** Gary Wong

1400 **Possible Involvement of Chemokines and Proinflammatory Cytokines in the Pathogenesis of Pulmonary Disease in Zaire Ebola Virus-Infected Pigs -** Charles Nfon

1420 **Genotyping of Anthrax Isolates from Disease Outbreaks in Canada -** Kingsley Amoako

1440 **A California Bighorn Sheep (*Ovis Canadensis californiana)* Lamb Mortality Investigation in an East Fraser River Herd, BC, Canada** - Jason Struthers

1500-1530 ***Break, exhibits*** *– Theatre Lobby and Orientation Room*

1530 **A Case of Equine Granulocytic Anaplasmosis** – Joan Bourque

1550 **SVD Testing, Why React?** - Kate Hole

1610 **Swine Vesicular Disease Virus Empty Capsid Particles: Characterization and Its Application as an Alternative Antigen for Serological Diagnosis** - Wanhong Xu

1630 **Development of Monoclonal Antibody against Foot-and-Mouth Disease Virus Asia1 and Application in Rapid Virus Detection by Lateral Flow Immunochromatographic Strip Test** – Ming Yang

1700 – **Tuesday session is adjourned**

1700-1900 - **National TSE Laboratory Network Meeting** (by invitation) – Main Boardroom

1730 – **Informal social get-together – King's Head Pub, 120 King Street (204-957-7710)**
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Diagnostic Developments for High Consequence Zoonotic Animal Pathogens

Hana Weingartl

National Centre for Foreign Animal Disease, Canadian Food Inspection Agency
Winnipeg, MB

Hendra virus (HeV) and Nipah virus (NiV) form a separate genus *Henipavirus* within the family *Paramyxoviridae*, Both viruses emerged from their natural reservoir *Pteropus* bats during the last decade of the twentieth century in Malaysia and Australia, respectively, causing severe disease in humans, horses and swine, and infecting a number of other mammalian species.

Rift Valley fever virus (RVFV), genus *Phlebovirus*, family *Bunyaviridae* is an arbovirus which undergoes enzootic and epizootic-epidemic transmission cycles, with *Aedes spp* of mosquitoes being the primary reservoir. The virus, endemic to Africa and Arabian Peninsula, can cause severe disease in humans, and severe often 100% fatal disease in newborn ruminants as well as high mortality in pregnant adult ruminants.

Ebola viruses belong to the family *Filoviridae,* genus *Ebola virus*. Those endemic to Africa cause severe hemorrhagic fever with frequent fatal outcome in humans and other simian primates. In 2009, Reston Ebola virus (REBOV), was isolated in Philippines from swine co-infected with porcine reproductive and respiratory syndrome virus, with antibodies against EBOV detected in pig farmers. REBOV did not cause disease in experimentally infected piglets, however the Zaire-EBOV (ZEBOV) can infect pigs, cause disease, and transmit to in-contact animals.

All three groups of viruses are considered high consequence pathogens, and require work in high (zoonotic agriculture level 3 or level 4) containment, also due to their high case fatality rate following human infection and the lack of effective vaccines or therapy.

One of the roles of Special Pathogens Unit at the NCFAD, CFIA is development of diagnostic capability for these agents. Main hindrance in development of assays for exotic agents requiring work in a high level of containment is availability of positive control samples, and limited knowledge of pathogenesis in the target host. Multiple experimental inoculations of animals were conducted to determine sampling strategy for each agent, as well as to generate at least minimal number of samples for evaluation of a diagnostic performance of individual tests. To date virus isolation, virus neutralization and real time RT-PCR assays were established for Hendra, Nipah, RVF and Ebola viruses. The Pathology Unit at NCFAD developed immunohistochemical assays for detection of the viruses in fixed tissues. The IHC and rRT-PCR are suitable for use in the field as well as in low containment laboratories. Development of recombinant antigen based ELISAs for antibody detection in low containment is more complex, as is the virus-host interaction on the organism level. Number of recombinant antigens and monoclonal antibodies were developed to assist in development of the above assays. In case of Nipah virus this lead also to development of a pen-side test. Dynamics of antibody development has to be determined for each specific agent in the target species to understand the possibilities for DIVA- compatible test development, and to evaluate diagnostic sensitivity of recombinant ELISAs. The later could be adversely affected by diagnostic specificity, especially in animals sick with other infectious organisms. Diagnostic specificity was estimated based on minimum of 300 serum samples tested per species per agent.

Diagnostic Developments for High Consequence Human Pathogens

Gary Kobinger

National Microbiology Laboratory, Public Health Agency of Canada
Winnipeg, MB

The impact of rapid diagnostic development on responses to Biosafety Level 4 infectious disease outbreaks and infection control. Infectious disease outbreaks involving BSL-4 viruses can be devastating for the socio-economical environment of affected communities. Rapid detection of these infectious agents is paramount to optimize coordination of outbreak responses and infection control measures. Substantial challenges have to be addressed when responding to highly virulent infectious agents such as viral hemorrhagic fever viruses (VHFs) including safe and efficient management of diagnostic facilities including field laboratories when performing real-time diagnostic. This presentation will review and discuss some of these challenges, concomitant solutions and how if affect infection control. With the constantly growing human population, infectious disease outbreaks will possibly amplify in frequency and size. Lessons learned in small VHFs outbreaks are of value for safe and efficient management of larger events and importantly for a better understanding of the animal-human interface and how it impact infection control.

International Twinning of Reference Laboratories and Harmonization of Diagnostics

John Pasick

National Centre for Foreign Animal Disease, Canadian Food Inspection Agency
Winnipeg, MB

As Canada's National Reference Laboratory for foreign animal diseases as well as an OIE Reference Laboratory for classical swine fever and avian influenza, the National Centre for Foreign Animal Disease has been actively involved in a number of initiatives aimed at diagnostic harmonization both regionally and internationally. NCFAD participates annually in international inter-laboratory comparison tests for avian influenza, Newcastle disease, bluetongue, foot-and-mouth disease, African swine fever and classical swine fever. As part of the North America Security and Prosperity Partnership (SPP) initiative, a North American Animal Health Laboratory Network was established whose aim is to harmonize the diagnostic capabilities for select diseases. NCFAD involvement in SPP has focused on harmonization diagnostic assays for foot-and-mouth disease, avian influenza, Newcastle disease and classical swine fever. These activities have been supplemented by initiatives supported by Procinorte, the Cooperative Program in Agricultural Research and Technology that facilitates cooperation among the three countries of IICA's Northern Region (Canada, USA and Mexico). Lastly, we are in the process of completing an OIE Twinning Project with LNDV-ICA Colombia that is providing technical support for the diagnosis and control of avian influenza and Newcastle disease.

Sheep and goat pox viral diagnostics and vaccine development collaboration

Shawn Babiuk[1,2], Timothy Bowden[3], David Wallace[4], Arshad Mather[4], Volker Gertds[5], Lorne A. Babiuk[6]

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Capripoxvirus are the cause of sheep pox, goat pox and lumpy skin disease of cattle. These diseases are of great economic significance to farmers in Africa and Asia where sheep and goat pox are endemic and in Africa where lumpy skin disease is endemic. These diseases are foreign animal diseases in North America and are a major constraint to international trade in livestock and their products. At NCFAD together with several different partners (CSIRO, Australia and ARC, South Africa) a wide array of modern diagnostic tests have been developed for capripoxvirus. These tests include the evaluation of suitable cell lines to propagate capripoxvirus for virus isolation, real-time PCR, serology, and the development of monoclonal antibodies for immunohistochemistry. To evaluate the diagnostic tests, sheep, goats and cattle were experimentally infected with capripoxvirus and samples were collected at specific time points. Currently together with partners from the University of Alberta, Vaccine & Infectious Disease Organization (University of Saskatchewan) and ARC (South Africa), we are attempting to develop a single vaccine to simultaneously protect against capripoxvirus, peste des petits ruminants and Rift Valley fever. While commercial vaccines are available against these pathogens, the cost of providing large-scale vaccination against these multiple diseases is more expensive then administration of a single vaccine to protect against multiple diseases. Therefore, the development of an affordable vaccine that protects against a variety of infectious agents would be of significant benefit to the livestock industry in Africa. The protective antigens of Rift Valley fever and peste des petits ruminants will be cloned into a lumpy skin disease virus vector, which has been previously found to elicit protection against other capripoxvirus (sheep and goat pox). Furthermore, previous studies have demonstrated Rift Valley fever glycoproteins, as well as the haemagglutinin protein of peste des petits ruminants can be expressed individually in a capripoxvirus vector, conferring protective immunity in both cases. Therefore, the expression of the protective antigens for Rift Valley fever and peste des petits ruminants in lumpy skin disease virus can potentially induce immunity against peste des petits ruminants, Rift Valley fever, sheep and goat pox in sheep and goats as well as lumpy skin disease and Rift Valley fever in cattle.

Development of vaccines for the prevention of viral diseases in livestock in Africa

Hani Boshra[1], Thang Truong[1], Charles Nfon[1], Shawn Babiuk[1,2]

[1]National Centre for Foreign Animal Disease, Canadian Food Inspection Agency, Winnipeg, MB, Canada, [2]Department of Immunology, University of Manitoba, Winnipeg, MB, Canada

In sub-Saharan Africa, agriculture accounts for a significant portion of economic activity; as livestock has enabled large segments of African society to emerge from poverty by providing sustainable levels of income. Although advances in the rearing of livestock has accounted for increased output, infectious diseases continue to pose a significant threat to livestock production, especially to small-scale farmers. While commercial vaccines are available against certain pathogens, the cost of providing large-scale vaccination against multiple diseases is a cost-prohibitive factor for many farmers in Africa. Therefore, the development of an affordable vaccine that protects against a variety of infectious agents would be of significant benefit to such farmers and to the livestock industry in Africa. One possible strategy for developing a multivalent vaccine against 5 endemic viral diseases in Southern Africa would include: lumpy skin disease virus, sheep and goat pox virus, Rift Valley fever virus and peste des petits ruminants virus. The protective antigens from Rift Valley fever virus and peste des petits ruminants virus will be cloned into a lumpy skin disease virus vector, which has been previously found to elicit protection against the other capripoxviruses sheep and goatpox. Previous studies have demonstrated that Rift Valley fever glycoproteins, as well as the haemagglutinin protein of peste des petits ruminants virus can be expressed individually in an lumpy skin disease vector, conferring protective immunity in both cases. Therefore, the expression of the protective antigens for Rift Valley fever and peste des petits ruminants in lumpy skin disease virus can potentially induce immunity against peste des petits ruminants, Rift Valley fever, sheep and goat pox in sheep and goats as well as lumpy skin disease and Rift Valley fever in cattle. In addition, a potential vaccine for African swine fever will be studied. African swine fever is endemic in Africa and parts of Europe and there are currently no vaccines available for African swine fever. While some proteins from African swine fever virus have been previously found to be recognized by infected animal sera, these proteins have been limited to diagnostic uses. Since antibody mediated immunity alone does not protect against African swine fever the vaccine strategy is to use a porcine adenovirus that encodes antigens from African swine fever virus to induce both antibody and cellular immunity. These vaccine candidates will be constructed and evaluated first for immunogenicity. Following these studies the vaccine candidates will be evaluated for efficacy in pigs challenged with African swine fever virus.

Evaluation of cell substrates and the development of RT-PCR and real-time RT-PCR assays for peste des petits ruminants

Thang Truong[1], Hani Boshra[1], Charles Nfon[1], Shawn Babiuk[1,2]

[1]National Centre for Foreign Animal Disease, Canadian Food Inspection Agency, Winnipeg, MB, Canada, [2]Department of Immunology, University of Manitoba, Winnipeg, MB, Canada

Peste des petits ruminants (PPR) is a viral disease of sheep and goats, characterized by fever, nasal discharge, cough, diarrhoea and death. The geographic distribution of peste des petits ruminants includes Africa and the Middle East as well as some parts of Asia including India and Pakistan. PPR virus is a member of the genus *Morbillivirus* in the family *Paramyxoviridae*, closely related to measles, rinderpest and canine distemper viruses. A stock of Malig-Yemen PPR virus, previously stored for 25 years at -70°C was amplified with three passages on Vero E6 cells. Virus amplification was assessed by evaluation of cytopathic effect. Further characterization of PPR virus growth was evaluated on Vero E6, primary lamb kidney, OA3.Ts and OA4.Ks cells. Specific RT-PCR and real-time RT-PCR assays were developed for PPR virus. These assays were used to characterize the growth of PPR in the different cell lines. Following these studies, peste des petits ruminants virus infection will be evaluated in sheep and goats to evaluate pathogenesis and develop a peste des petits ruminants virus challenge model.

Nipah virus infects specific subsets of porcine peripheral blood mononuclear cells

Beata Stachowiak [1,2] and Hana M. Weingartl [1,2]

[1]National Centre for Foreign Animal Disease, Canadian Food Inspection Agency, Winnipeg, MB, Canada, [2]Department of Medical Microbiology, Faculty of Medicine, University of Manitoba, Winnipeg, MB, Canada

Nipah virus (NiV), a zoonotic paramyxovirus, is highly contagious in swine, and can cause fatal infections in humans following transmission from the swine host. The main viral targets in both species are the respiratory and central nervous systems, with viremia implicated as a mode of dissemination of NiV throughout the host. The presented work focussed on the role of peripheral blood mononuclear cells (PBMC) in the viremic spread of the virus in the swine host. B lymphocytes, CD4-CD8-, as well as CD4+CD8- T lymphocytes were not permissive to NiV, and expansion of the CD4+CD8- cells early post infection was consistent with functional humoral response to NiV infection observed in swine. In contrast, significant drop in the CD4+CD8- T cell frequency was observed in piglets which succumbed to the experimental infection, supporting the hypothesis that antibody development is the critical component of the protective immune response. Productive viral replication was detected in monocytes, CD6+CD8+ T lymphocytes and NK cells, by recovery of infectious virus in the cell supernatants. Virus replication was supported by detection of the presence of structural N and the non-structural C proteins or by detection of genomic RNA increase in the infected cells. Infection of T cells carrying CD6 marker, a strong ligand for the activated leukocyte cell adhesion molecule ALCAM (CD166), was highly expressed on the microvascular endothelial cell of the blood-air and the blood-brain barrier, which may explain NiV preferential tropism for small blood vessels of the lung and brain.

A new novel pen-side strip test for the detection of Nipah virus in swine

Mingyi Li [1], Yohannes Berhane[1], Jody Berry[2], Greg Smith[1], Hana Weingartl[1,3]
[1]National Centre for Foreign Animal Disease, Canadian Food Inspection Agency, Winnipeg, MB
[2]Molecular Immunology Division, Research & Development, Cangene Corporation
[3]Department of Medical Microbiology, University of Manitoba, Winnipeg, MB

Nipah virus (NiV), member of the genus *Henipavirus*, under the family *Paramyxoviridae*, was identified as the causative agent for the outbreak of human respiratory syndrome and encephalitis in Malaysia in 1998. Several hundred human cases have been confirmed with a fatality rate as high as 92%, including outbreaks of in Singapore, India, and Bangladesh. Pigs served as the intermediate host for the transmission of NiV from fruit bat to human in 1998, which was not aware of for two years, due to very little signs of illness in swine. Early identification of NiV infection in swine has been a very interesting topic and very important step for the control of virus spreading. However, most of the tests developed for the diagnosis of this virus infection rely on the BSL4 facility and highly trained personnel. Lateral flow (pen-side) strip test was developed and used for the diagnosis of pregnancy in 1980s. It is simple to perform and quick to see the result with naked eyes, without the need of specialized equipments and trained personnel. Here we describe a newly developed pen-side strip test for the detection of NiV in swine nasal swab or nasal wash samples when available. A monoclonal antibody against the nucleoprotein of NiV was developed and used for the assembly of the test strips. The test can detect viral protein as early as 4 days post infection (dpi) in nasal swabs or 1 dpi in nasal wash samples from our experimentally infected pigs. It was found that it detected more positive samples with stronger signals from the nasal wash samples, compared to nasal swabs, therefore the former being the ideal sample for this test. When nasal wash samples were evaluated, the test achieved similar sensitivity and specificity, in comparison to virus isolation and specific real-time RT-PCR for NiV-N. With the development of this test, it is possible for a farmer or veterinarian to check the presence of NiV pathogens in suspicious animals immediately, before sending samples for further confirmation. The simplicity of the test warrants its use as a good herd-status tool.

Immune Parameters Correlating with Protection against Ebola Virus Infection in Rodents and Nonhuman Primates

Gary Wong[1, 2, *], Jason S. Richardson[1, *], Stéphane Pillet[1], Ami Patel[1, 2], Xiangguo Qiu[1], Jeff Hogan[3], Yi Zhang[4], Ayato Takada[5], Heinz Feldmann[2, 6] and Gary P. Kobinger[1, 2, 7]

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[*] The authors contributed equally to the work.

Ebola virus (EBOV) is a pathogen that causes severe hemorrhagic fever in susceptible hosts. There are currently no licensed EBOV vaccines or treatments available but several experimental vaccines have been successful at protecting rodents and nonhuman primates (NHPs) from a lethal dose of Zaire ebolavirus (ZEBOV). The objective of this study was to evaluate humoral and cellular immune responses correlating with survival following lethal challenge with ZEBOV in rodents and NHPs.

Several knockout mouse lines with an impaired ability to generate a normal adaptive immune response were vaccinated and challenged with a lethal dose of ZEBOV. Immune responses in survivors and non-survivors were first characterized in the mouse animal model, and then further evaluated in both the guinea pig and NHP animal models.

The use of knockout mice revealed that vaccine-induced protection against ZEBOV is mainly mediated by B-cells with possible involvement of NK and CD4+ T-cells. Levels of total immunoglobulin G (IgG) specific to the ZEBOV glycoprotein (ZGP) was shown to correlate with survival from ZEBOV challenge in both guinea pigs and NHPs. A specific T-cell response was also found to confer a significant advantage for host survival early after ZEBOV exposure.

These results highlight the relevance of total ZGP-specific IgG levels as a meaningful correlate of protection which could predict survival against a lethal dose of ZEBOV with ≥99.9% confidence in vaccinated mice, guinea pigs and NHPs. To combat ZEBOV infection post-exposure, a complex coordination between different arms of the immune response seems necessary to limit disease progression and to develop protective immunity.

Possible Involvement of Chemokines and Proinflammatory Cytokines in the Pathogenesis of Pulmonary Disease in Zaire Ebola Virus-Infected Pigs

Charles Nfon[1], Anders Leung[2], Greg Smith[1], Carissa Embury-Hyatt[1], Gary Kobinger[2,3,4] and Hana Weingartl[1,3]

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Ebola viruses (EBOV) are filamentous single-stranded RNA viruses of the family *Filoviridae*. Zaire EBOV (ZEBOV) causes severe haemorrhagic fevers in humans and non-human primates (NHPs) with high fatality rates. In Contrast, Reston EBOV (REBOV), the lone strain found outside Africa, is only lethal to NHPs and not to humans. REBOV was isolated from pigs in the Philippines and subsequent experiments confirmed the susceptibility of pigs to both REBOV and ZEBOV with predilection for the lungs. However, only the latter caused severe lung pathology leading to respiratory distress. To further elucidate the mechanisms for lung pathology, microarray analysis of changes in gene expression was performed on lung from ZEBOV-infected pigs. Furthermore, systemic effects were monitored by looking at changes in peripheral blood leucocyte subsets and systemic cytokine responses. Following challenge via the oronasal route, ZEBOV replicated mainly in the respiratory tract, with the main clinical signs being rapid and difficult breathing. Lungs showed severe inflammation and had an abundance of neutrophils, macrophages and lymphocytes. Genes for proinflammatory cytokines, chemokines and acute phase proteins, known to attract immune cells to sites of infection, were upregulated in the lungs, possibly accounting for the heavy influx of cells into this site. Systemic effects of ZEBOV included a decline in the proportion of monocyte/dendritic and B cells and an increase in blood proinflammatory cytokine levels. Serum IgM and neutralising antibodies were detected on day 5 and 6 post infection. In conclusion, a dysregulation/over-activation of the pulmonary proinflammatory response may play a crucial role in the pathogenesis of ZEBOV infection in pigs by attracting inflammatory cells to the lungs.

Genotyping of anthrax isolates from disease outbreaks in Canada

Kingsley Amoako, Kristen Hahn, Matthew Thomas, Timothy Janzen, Michael Shields and Noriko Goji.

Lethbridge Laboratory, Canadian Food Inspection Agency
Lethbridge, AB

The need to detect microbial agents during disease outbreaks has motivated molecular biologists to develop more highly discriminating assays to genetically characterize and potentially track these microorganisms. The routine utilization of a molecular approach for the identification of anthrax isolates will allow accurate identification of the isolates and will provide a more reliable correlation of possible differences in clinical outcome, virulence and epidemiology. In microbial epidemiology, information on the distribution of types is used for defining the source and route of infection, for studying the persistence and re-infection rates, clonal selection and microbial evolution. A number of DNA-based genotyping technology platforms are being actively explored to meet this challenge. A new generation of Genomics based technologies such as the Optical Mapping System (OMS) and Pyrosequencing have sufficient discrimination potential to be used for detection and typing of isolates with very low levels of genetic diversity and also for undisputable detection of new sub-types. The OMS is a novel de novo process that generates whole genome, ordered, restriction maps with no requirement for previous sequence information. Pyrosequencing technology is a relatively inexpensive, easy-to-use method that employs coupled enzymatic reactions to determine the nucleotide composition of a target DNA. In comparison to Sanger sequencing, Pyrosequencing technology is limited to short fragments. We explored these novel technologies for the genotyping of anthrax isolates from disease outbreaks in various parts of Canada and also in different animal species. We selected markers including Single Nucleotide Repeats and Canonical Single Nucleotide Polymorphisms and successfully applied Pyrosequencing to determine the genetic and phylogenetic relatedness among a select number of anthrax strains isolated from disease outbreaks in Canada. Using optical mapping technology as a high resolution whole genome analysis tool, we further characterized these disease outbreak isolates. The application of the OMS technology provided a capability to 1) compare anthrax isolates from multiple locations to confirm/negate same source strain, 2) perform map-based similarity clustering to precisely distinguish isolates, 3) characterize anthrax outbreak strains efficiently through genomic rearrangements, including inversions, insertions and deletions, and 4) characterize *Bacillus anthracis* whole genomes rapidly and cost-effectively with the potential to trace and link disease outbreak strains to source. The OMS and Pyrosequencing technologies enabled high resolution visualization of strain differences/similarities allowing for comparison of anthrax strains by characterizing genetic variation to distinguish closely related anthrax strains and accurately detail the genomic differences between isolates.

A California bighorn sheep (*Oviscanadensiscalifornia)* lamb mortality investigation in an East Fraser River herd, BC, Canada

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Chronic poor recruitment and lamb mortality was investigated in a California bighorn sheep herd from British Columbia's (Canada) interior in the summer of 2011. Daily monitoring of the herd from mid-June to mid-July identified coughing and diarrhea which increased in prevalence over time with approximately 32-39% of lambs being affected near the end of the study period. Necropsies of two euthanized sick lambs and one naturally deceased lamb revealed severe bronchopneumonia. *Mycoplasma ovipneumoniae* was determined to be a significant pathogen based on characteristic histological lesions and its identification using PCR. Other bacteria isolated from lungs, pharynx and tympanic bullae included: *Bibersteiniatrehalosi, Pasteurellaspp, Mannheimiahaemolytica,* and *Streptococcus suis.* Although lungworm (*Protostrongylus*spp)was suspected to be a contributing cause of pneumonia there were no compatible pulmonary histological lesions and only one nematode was found at necropsy. Low counts of lungworm larvae in lamb and adult feces (n=77, median=4 LP5g, range=0-213 LP5g) collected during the summer supported this result. Our findings suggest *Mycoplasma ovipneumoniae* is a major cause of morbidity in these lambs and we hypothesize additional factors, such as secondary bacteria, inclement weather and predation of sick lambs, results in high lamb losses in some years. Further research is required to confirm these findings and to determine the relative importance of additional factors on poor recruitment.Although the disease ecology in this herd was not an investigative priority at the time, there is speculation that historical contact with domestic sheep (*Ovisaries)* may have been an important instigating factor.Western jurisdictions have frequently associated epizootics of bighorn sheep pneumonia as occurring from contact with domestic sheep, in which their role as infectious disease vectors have been experimentally demonstrated to be credible. This investigation describes a situation suggestive of conflict at the wildlife-domestic animal interface and supports the need forthe development of new tools for its mitigation.

Equine Granulocytic Anaplasmosis in a New Brunswick Horse

Joan Bourque and James Goltz

New Brunswick Provincial Veterinary Laboratory
Fredericton, NB

On November 15, 2011, blood samples from a 12 year old Paint gelding horse were submitted to the New Brunswick Provincial Veterinary Laboratory for a complete blood count, differential nucleated cell count and equine clinical chemistry profile. The clinical history indicated that the horse had been progressively becoming more exercise intolerant and exhibiting diminishing energy levels during the previous week, and had a bilateral nasal discharge, serous on one side and serohemorrhagic on the other. Laboratory testing revealed a pronounced leukopenia and neutropenia, accompanied by mild anemia, and slight hypoalbuninemia, bilirubinemia and hyponatremia. A number of neutrophils in Wright's-Giemsa stained blood smears were found to contain basophilic intracytoplasmic structures morphologically consistent with the morulae of *Anaplasma phatocytophilum*. PCR testing at the National Microbiology Laboratory in Winnipeg confirmed the diagnosis of Equine Granulocytic Anaplasmosis. The horse completely recovered following a one week course of intravenous tetracycline injections. This represents the first known case of this tick-borne disease in a horse in New Brunswick and the third reported equine case in Canada. This case emphasizes the importance of having a national animal health network to facilitate the sharing of information on emerging diseases, and exemplifies how veterinary laboratories are an integral part of One Health surveillance for emerging zoonotic diseases.

Why React?

Kate Hole, Zhidong Zhang

National Centre Foreign Animal Diseases, Canadian Food Inspection Agency
Winnipeg, MB

Porcine samples are tested pre-export for the presence of antibodies to Swine Vesicular Disease (SVD) by request from certain countries. The standard prescribed test used is a competitive ELISA developed by Brocchi et al. using their monoclonal antibody (MAb) 5B7 and referred to as the MAC-ELISA. In Europe, a higher cut-off value is used as the disease has been present and has been observed as mild and at sub-clinical levels. Based on European numbers, results from a small proportion (0.25%-0.45%) of sera from normal pigs are borderline or positive by the MAC-ELISA. In Canada where there is no disease, a lower cut-off value is used which may lead to an even higher level of observed false positives. False positives are further tested by virus neutralization test (VNT) to try to show their status as reactors only. Unfortunately, on average, only 50% of the cELISA false positive samples will be negative by VNT which leads us with the question of why? Consistent problems with a higher percentage of false positives, termed singleton reactors appear to be associated with specific geographic regions across Canada. The factors responsible for these reactors are unknown. Serological cross-reactivity with SVD virus might arise due to infection with another, as yet unidentified, picornavirus or may be due to other non-specific factors present in the serum. Currently tests are under way to try to ascertain the cause of these reactors whether it is a response to an unknown viral infection, potentially related to inoculation schedules or something else entirely. There appears to be a definite age related effect. Results will be discussed.

Swine Vesicular Disease Virus Empty Capsid Particles: Characterization and Its Application as an Alternative Antigen for Serological Diagnosis

Wanhong Xu, Melissa Goolia, Ming Yang, Zhidong Zhang

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Winnipeg, MB

Swine vesicular disease virus (SVDV) causes a vesicular disease in pigs. The importance of the disease is due to its indistinguishable clinical signs from those caused by foot-and-mouth disease, and the presence of the disease may prevent international trade of pigs and pig products. Currently, diagnosis of SVDV infection solely relies on laboratory investigation. Serological tests for SVD are key assays for disease surveillance or export certification. The enzyme-linked immunosorbent assay (ELISA) requires preparation of inactivated SVD virus as an antigen, which is often cumbersome and time-consuming process conducted in a containment level 3 laboratory. To overcome these disadvantages, a recombinant virus-like particle of SVDV was produced by utilizing a Bac-to-Bac baculovirus expression system. Analyses of empty capsid particles by electron microscopy, Western blotting, and mass spectrometry confirmed that the empty capsid particles morphologically resemble those of authentic viruses but consist of only uncleaved VP0, VP1 and VP3. The antigenicity of the empty capsid particles was proved by an immunofluorescent assay. The application of the recombinant empty capsid particles as an antigen was evaluated and compared with those of inactivated authentic viruses in a competitive ELISA as well as isotype-specific ELISAs. The results showed that the recombinant viral-like particle antigen is comparable with binary ethyleneimine (BEI) inactivated whole virus antigen in terms of their diagnostic sensitivity and specificity. In conclusion, the recombinant SVDV empty capsid particle antigen offers a safe valuable alternative to virus antigen for diagnostic assays.

Development of monoclonal antibody against foot-and-mouth disease virus Asia 1 and application in rapid virus detection by lateral flow immunochromatographic strip test

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To develop a rapid detection method for the foot-and-mouth disease virus (FMDV) serotype Asia 1, two specific monoclonal antibodies (mAbs) against FMDV serotype Asia 1 were produced and shown to react specifically with FMDV serotype Asia 1 without cross-reactivity with other FMDV serotypes.

A lateral flow immunochromatographic strip test for FMDV serotype Asia1 antigen detection was then developed using these two mAbs as the capture mAbs. A serotype independent mAb was selected and used as the detection mAb in order to provide a foundation for the development of a multiple serotype strip test in the future. A new generation of the RapidAssay Device (gRAD) was also used for the first time. The strip test can specifically detect the FMDV Asia 1 virus, but not other FMDV serotypes or other vesicular disease viruses (n=143). The test was able to identify all tested FMDV Asia1 strains (n=11). The sensitivity of this strip test was comparable with the double antibody sandwich ELISA for viral antigen detection. All vesicular fluid and epithelium (the tongue, the interdigital area and coronary band on the feet) samples collected from pigs experimentally infected with Asia1 Pak 20/2003 were identified as positive by the strip test. However, the strip test is not suitable for testing samples of nasal swaps and serum samples.

Due to its ability to produce rapid results and high specificity, the strip test would be useful to rapidly diagnose FMDV Asia1 in the field. In an endemic situation or during an epidemic, this would eliminate the procedure for sending suspicious samples to a laboratory for testing and reduce the delay in diagnosis.

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CAHLN-RCTLSA 2012 – Wednesday, June 6 Program

One Health - Emerging Diseases

Session Moderator: Shawn Babiuk

0830 **Experimental Challenge of Cattle with Classical and Atypical BSE: Clinical Presentation and Characterization of Neuropathology –** Catherine Graham

0850 **User-Friendly Microarray for Porcine Respiratory Disease Complex –** Aruna Ambagala

0910 **Microarray Detection and Typing of Livestock and Poultry Viruses –** Oliver Lung

0930 **Diagnosis of Swine Respiratory Infectious Agents using a Nano-Volume Quantitative PCR Platform –** Philippe Garneau

1000-1030 ***Break***

1030 **Development of Multiplexed Fluorescent Microsphere Immunoassay for Detection of Antibodies Against Avian Influenza Virus (NP, H5 and H7) and Newcastle Disease Virus (NDV) –** Marsha Leith

1050 **Molecular and Antigenic Characterisation of Reassortant H3N2 Viruses from Turkeys with a Unique Genetic Constellation –** Tamiko Hisanaga

1110 **Evaluating the Role of Internal Influenza Virus Proteins (PB2, PB1, PA, NP, M and NS) in Protecting Chickens Against Lethal H5N1 Challenge** – Sandra Diederich

1130 **Presentation of** CAHLN-RCTLSA Graduate Student Award

1200 *Adjourn*

Experimental challenge of cattle with classical and atypical BSE; Clinical presentation and characterization of neuropathology

Catherine Graham[1], Michel Levy[2], Ed Pajor[2], Garth McGregor[1], Rheana Flitton[1], Stefanie Czub[1*]

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Classical, or C-type, bovine spongiform encephalopathy (BSE) has been extensively described in the literature. Recently, two novel forms of BSE, termed atypical BSE, have been reported in a number of countries. These new forms show differences in the biochemical characteristics of the prion protein and, where reported, tend to occur in aged animals but descriptions of clinical presentation are incomplete or absent. In addition, distribution of histopathologic changes and PrP^{Sc} accumulation shows differences between the classical and atypical forms.

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Female Hereford/Angus cross calves were intracranially challenged at approximately 5 months of age with 1 ml of a 10% brain homogenate originating from Canadian field cases of BSE which had been previously classified as C-, L-, or H- type.

The animals were monitored during incubation period, and clinical disease is described using a standardized examination protocol. Incubation period, description and progression of clinical signs was recorded and videotaped for objective evaluation.

The animals were humanely euthanized after they developed definite and progressive clinical disease. Selected regions of the brain were tested using IDEXX-HerdCheck EIA, histopathology and immunohistochemistry.

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All L- and H- type atypical BSE challenged animals began to display signs of clinical disease at approximately 11 months post inoculation, and disease progression was slow but constant until animals were euthanized. Clinical signs in all atypical BSE inoculated animals included hesitation at doors and gates, spontaneous muscle fasciculations, and sensitivity to touch. Teeth grinding and excessive salivation were occasionally noted. Animals with L-type BSE were very anxious and showed high levels of sensitivity to hand movement. One H-type animal showed periods of somnolence. Both H-type inoculated animals go down during handling and have difficulty rising and show sensitivity to movement around their head and neck area, but to a lesser degree than the L-type BSE inoculated animals.

C-type challenged animals began to display clinical abnormalities at approximately 18-20 months post inoculation. Progression of clinical disease was typically slow and intermittently displayed during the initial stages. Clinical signs in animals inoculated with C-type BSE were as previously reported in the literature.

The spectrum of clinical signs for all three types of BSE examined is similar. Incubation period is shorter for H- and L-type BSE as compared with C-type. Once clinical signs present, progression is slow but relentless in atypical BSE, and more insidious in classical BSE. A summary of clinical signs presented in the 3 different types of BSE will be presented, and video of clinical disease will be displayed. Neuropathologic comparison of all three types of BSE will be described and detection of PrP^{Sc} by immunohistochemistry and rapid test will be presented.

User-friendly Microarray for Porcine Respiratory Disease Complex

Aruna Ambagala[1], Sam Ohene-Adjei[1], Tomy Joseph[2], Robin King[3], Tara Furukawa-Stoffer[1], Anthony Erickson[1], Anne Beeston[1], Oliver Lung[1]

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Porcine Respiratory Disease Complex (PRDC) is a mutli-factorial disease that can involve several bacterial and viral pathogens. PRDC has a significant impact on production efficiency and is the most important health concern of pig producers. Current methods for detection of porcine respiratory pathogens are inefficient and time consuming, often requiring independent tests for each pathogen. No diagnostic assay exists for simultaneous detection of major viral and bacterial pathogens of PRDC. DNA microarrays are ideal for highly multiplexed detection and typing of pathogens.

Here we describe development of a user-friendly DNA microarray for the simultaneous detection and differentiation of four major porcine respiratory viruses (porcine reproductive and respiratory syndrome virus, swine influenza virus, porcine respiratory corona virus and porcine circovirus 2) and four major bacterial species (*Mycoplasma hyopneumoniae*, *Pasteurella multocida*, *Salmonella enterica serovar Choleraesuis and Streptococcus suis*) associated with the PRDC. Using available sequence data, we created a large sequence database of the target viral and bacterial pathogens, and the database was used to design primers and probes specific for each pathogen species. Multiple probes were designed for each target species and also for an internal control. We have successfully developed a multiplex PCR that can simultaneously amplify all four viral and four bacterial targets, and the internal control. All targeted pathogens and the internal control were accurately identified when the designed probes were tested against targeted pathogens on the automated NanoChip-400 electronic microarray platform. The assay will be validated using clinical samples to compare diagnostic sensitivity and specificity.

Microarray detection and typing of livestock and poultry viruses

Oliver Lung[1], Dirk Deregt[1], Dalibor Hodko[2], John Pasick[3], Don King[4], Zhidong Zhang[3], Tara Furukawa-Stoffer[1], Anne Beeston[1], Sam Ohene-Adjei[1], Kim Burton-Hughes[1], Mathew Fisher[1], Kristen, Hahn[1], Cody Buchanan[1], Aruna Ambagala[1]

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Microarrays can be a cost-effective tool for multiplexed detection and typing of infectious agents. However, conventional microarrays generally require capture probes to be pre-printed, rely on slow passive hybridization that take hours to complete, and there are considerable demands on user-handling time and expertise. In this study, PCR and associated microarray assays for multiplexed detection of high consequence bovine, swine, and poultry viruses were developed for a user-friendly electronic microarray platform that utilizes rapid electrophoretically-driven hybridization and automates capture probe printing and microarray processing. The bovine assays target viruses that include foot-and-mouth disease virus (FMDV), vesicular stomatitis virus (VSV), alcelaphine herpesvirus-1 (AlHV-1), rinderpest virus (RPV) and bluetongue virus (BTV); the swine assay targets viruses that include FMDV, classical swine fever virus (CSFV), African swine fever virus (ASFV), swine vesicular disease virus (SVDV) and vesicular exanthema of swine virus (VESV) and the avian assays target avian influenza virus (AIV) and Newcastle disease virus (NDV). The bovine assays accurately detected a panel of 65 bovine viruses that included viral strains representing all 7 FMDV serotypes, both VSV serotypes and multiple BTV serotypes. The avian assays accurately detected and typed 63 avian viruses that included 41 AIV strains representing all 16 H types and 9 N types, and 22 high and low pathogenicity NDV strains. The assays detected viruses in spiked biological samples and in clinical samples from experimentally infected animals. Results on the transfer of the assays to fully automated “sample-to-answer” instrument that require no user intervention after sample addition will also be presented.

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

Philippe Garneau[1], Véronique Allard[2], Tony Sabetti[3], Donald Tremblay[2], Carl A. Gagnon[1], Marie Archambault[1], Josée Harel[1]

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We are developing a novel diagnostic approach for simultaneous identification and quantification of swine respiratory infectious agents bringing losses to the industry. The Porcine Reproductive and Respiratory Syndrome Virus (PRRSV) in particular cause important economical losses to swine producers. The virulence of PRRSV is associated with multiple factors including other viral pathogens such as Circovirus, Influenza virus and bacterial infections.

The main goal of this project is to develop a comprehensive and efficient identification and characterization diagnostic approach for viral and bacterial agents responsible for swine morbidity and mortality. We propose to use the OpenArray® technology from Life Technologies. This platform, based on nanofluidic q-PCR technology, allows the identification of up to several hundred agents simultaneously.

We have reached the first milestone of our study. We selected the target respiratory infectious agents as well as identified representative sequences. Using bioinformatic analysis, we designed DNA primers/probes compatible with the OpenArray® q-PCR technology operating parameters. The specificity and sensitivity parameters characteristic of these sets were evaluated using previously characterized samples. When their real time qPCR results met the platform's efficiency requirements, the primer/probe sets were kept, otherwise new sets were designed. The first quantification laboratory tests on the sets were conclusive.

The capabilities and performance characteristics of the system are being evaluated. The development of rapid and accurate tools for diagnosis is important and contributes to improve animal infectious disease eradication or control programs. It is our hope it will contribute to support a high productivity program of the swine industry.

Development of multiplexed fluorescent microsphere immunoassay for detection of antibodies against avian influenza virus (NP, H5 and H7) and Newcastle disease virus (NDV)

Marsha Leith, Yohannes Berhane, Matthew Suderman and John Pasick

National Centre for Foreign Animal Disease, Canadian Food Inspection Agency
Winnipeg, MB

Outbreaks due to virulent NDV and to avian influenza viruses of H5 and H7 subtypes are reportable diseases to the World Organization for Animal Health (OIE) and both viruses cause economically devastating outbreaks in domestic poultry. Currently, serologic monitoring of ND in commercial poultry is accomplished by using a variety of commercial enzyme-linked immunosorbent assay (ELISA) kits and by hemagglutination inhibition (HI) assays. Surveillance of avian influenza (AI) in Canada is accomplished by detecting antibodies to group specific antigens (NP and Matrix) by using agar gel immunodiffusion (AGID) test or by ELISA. These currently used serological methods have some limitations: the AGID test is time intensive, not quantitative and AGID positive samples must be further subtyped by hemagglutination inhibition (HI) and neuraminidase inhibition (NI) tests. Both HI and NI tests are labour and time intensive, require subtype-specific antibodies and viruses and can only be done in BSL-3 laboratories. Competitive ELISA tests using NP as antigen are sensitive and quantitative, but positive samples must also be subtyped by HI assay. These limitations can delay the results and hence slow down the response to outbreaks. The goal of this project was to develop an assay that allows detection and subtyping of AI as well as differential diagnosis from Newcastle Disease. To accomplish this goal we have developed a quadriplex fluorescence microsphere immunoassay (FMIA) based on Luminex technology. In this assay, magnetic microspheres, with specific identifying fluorescent properties, are conjugated to AIV-NP, H5, H7 and NDV-NP baculovirus-expressed recombinant proteins and additional HA and NA antigens can be added as required. These microspheres act as a solid phase for the detection and quantification of antibodies in serum samples. This multiplexing capability allows the detection and subtyping of antibody response in the same platform using minimal sample volume within a short span of time. This allows the labs to report results with a short turn around so that authorities in the field can take the necessary measures.

Molecular and Antigenic characterisation of reassortant H3N2 viruses from turkeys with a unique genetic constellation

Yohannes Berhanne[1], Tamiko Hisanaga[1], Helen Kehler[1], Kathy Handel[1], Wanhong Xu[1], Davor Ojkic[2] and John Pasick[1]

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Triple reassortant (TR) H3N2 influenza viruses have been implicated in decreased egg production from breeder turkeys. In 2011, an outbreak in a commercial operation comprising three differing geographic locations was diagnosed with a drop in egg production from TR H3N2 viruses. The 8 gene segments from each of these viruses was determined, indicating the initial virus was entirely of TR H3N2 lineage, whereas the subsequent viruses displayed a unique mixture of 2009 pH1N1 gene segments as well as those of TR H3N2. To our knowledge this is the first report of TR H3N2/pH1N1 from domestic poultry. Phylogenetic analysis of the viruses of the HA and NA genes place the 3 viruses in 2 separate subclades within cluster IV of TR H3N2 viruses. Comparisons of each TR H3N2 virus with the prototype TR H3N2 cluster IV virus (A/Sw/ON/33853/2005) indicated 95% or 91% identity.

The hemagluttinin protein is an important target of the host immune response, with the majority of the immunodominant antigenic sites located on the globular head of the HA1 subunit. Turkey flocks from two of the farms had been vaccinated with a H3N4 oil emulsion vaccine prior to the outbreak. Comparisons of the HA1 subunit from the vaccinated flocks showed a total of 16 amino acid substitutions, whereas the non-vaccinated flock's isolated virus had a total of 19 amino acid substitutions with respect to the H3N4 vaccine strains antigenic sites. The ability of the field strains to infect vaccinated turkey flocks and cause a loss of egg production is likely due to changes at the globular head and has implications for future vaccine regimens.

Evaluating the role of internal influenza virus proteins (PB2, PB1, PA, NP, M and NS) in protecting chickens against lethal H5N1 challenge

Sandra Diederich, Yohannes Berhane, Carissa Embury-Hyatt and John Pasick

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Highly pathogenic avian influenza viruses (HPAIV) continue to circulate in domestic bird populations, resulting in devastating losses. Moreover, transmission of HPAI H5N1 or H7N7 viruses to humans raises the concern that these might evolve into a new human strain. In countries where the H5N1 is endemic, vaccination may be the best measure to reduce the impact of influenza virus outbreaks. Vaccines that are able to induce protective immunity to a range of influenza A virus subtypes are highly desirable. Recent studies in various animal models have demonstrated the role of hetero-subtypic immunity in protecting against different influenza A infections.

In this study, we investigated the impact of the internal influenza genes in protecting chickens against lethal H5N1 challenge. For this purpose a recombinant virus bearing the 6 internal genes of the HPAIV H7N3 (A/Ck/BC/CN007/2004) and the HA and NA genes of the HAPIV H5N1 A/swan/Ger/R65/2006 was generated using reverse genetics. This recombinant H5N1 virus (rH5N1) was shown to have an intravenous pathogenicity index of 2.3, which is characteristic for HPAIV. Specific pathogen free White Leghorn chickens were inoculated intranasally with the low pathogenic precursor of HPAI H7N3 A/Ck/BC/CN006/2004. This precursor differs from the HAPIV by the absence of a furin recognized cleavage site in the HA. Twenty-seven days following H7N3 infection, these chickens were challenged with 10000 plaque-forming units (PFU) of the rH5N1. Control chickens that were not previously infected with H7N3 exhibited clinical signs of infection beginning at day 3 post inoculation (DPI) whereas the challenged animals showed slightly less severe signs of illness starting at 5 DPI. However, there were no obvious differences in the microscopic lesions found in various tissues or virus shedding from oropharyngeal or cloacal routes. Supporting these findings, there was no significant difference in the survival rate of chickens from both groups with a slightly higher median time of survival in the pre-treated group (10 days versus 7 days). Associated with this prolonged survival of the H7N3 pre-infected/rH5N1 challenged birds, was the generation of H5-specific antibodies.

In this experimental set-up, immunization of chickens with a H7N3 virus did not induce robust protection against infection with a recombinant HPAI H5N1 virus that contained the H7N3 internal genes. We were therefore not able to prove the concept that influenza A virus internal genes are sufficient in providing cross-protection in chickens.

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