ABSTRACTS

PROCINORTE Animal Health Task Force

"Emerging Swine Pathogens that Pose a Threat to North America"

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Instituto Nacional de Investigaciones Forestales, Agrícolas y Pecuarias (INIFAP)

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Meeting Room: "*Sala de Usos Múltiples*"

ASF and CSF diagnostics and research at the National Centre for Foreign Animal Disease, Canadian Food Inspection Agency

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The Canadian Food Inspection Agency- National Centre for Foreign Animal Disease (NCFAD) Laboratory is one of the OIE Reference Laboratories for Classical Swine Fever (CSF) and the Canadian National Reference Laboratory for African swine fever (ASF). NCFAD provides state-of-the-art scientific expertise and technologies for the prevention, detection, control and reporting of foreign animal diseases, zoonotic infections and emerging diseases. In addition to containment level 2, 3, and 3Ag (for larger agriculture) facilities, NCFAD also operates a containment level 4 laboratory, which provides the ability to work safely with the most serious zoonotic viruses.

The Mammalian Diseases unit at the NCFAD provides diagnostic services to a number of high consequence swine diseases including classical swine fever and African swine fever. The diagnostic services include a wide range of molecular (real-time and conventional PCR, Sanger sequencing, genotyping), serological (ELISA, immunoblotting, immunoperoxidase tests), virological assays (virus isolation and characterization) and pathogenesis studies (in vivo animal experiments). Research activities include development and validation of new improved test methods, evaluation of commercial diagnostic kits, evaluation of emerging diagnostic technologies, evaluation of vaccines, and pathogenicity studies related to ASF and CSF. In addition the mammalian diseases unit provides ASF and CSF proficiency testing for the Canadian Animal Health Surveillance Network, and OIE member countries. The ongoing research activities and international collaborations related to ASF and CSF will be discussed in details.

Presenter: Manuel V. Borca

Title: Status of CSF research at ARS towards the development of novel marker vaccines

This talk will be centered on the research activities that have focused on the identification and characterization of CSFV genetic determinants of virulence and the use of the generated information towards designing live attenuated marker vaccine candidates.

The presentation will describe several approaches used to: a) identify genetic markers of virulence/attenuation in a highly virulent isolate of CSFV; b) modify genetically the CSFV genome (reverse-genetics); c) test genetically modified viruses for virulence and d) assess the protective efficacy of attenuated recombinant viruses.

This presentation will also display additional steps that were taken to introduce genetic markers into the CSFV genome that enable the distinction of vaccinated from naturally infected animals by means of serology (ELISA) as necessary DIVA-vaccine companion test.

Presenter: Consuelo Carrillo

Title: ASF Diagnostics Gap Analysis: GARA and EURL/OIE views and results

Emerging pathogens may include previously unknown disease-causing agents that may emerge due to novel host-pathogen combinations (i.e., SARS), pathogens with apparently increased virulence (i.e., hyper virulent Seneca virus A (SVA)), or previously known infectious agents that demonstrate a sudden and rapid increase in incidence and range (i.e., African swine fever virus (ASFV)). ASFV is a highly complex viral disease of swine and like SVA is an Emergent Swine Pathogen (ESP) contributing to one of the most pressing problems in animal heath, food security and public health. Resolution of the problem involves enhancing preparedness through strengthening early detection, surveillance and response capabilities coordination of regional activities, fluency and transparency of communication, and harmonization of methods used in decision making processes.

ASFV is an emerging threat to swine industry; pork is one of the most critical protein sources for developing countries and a rapidly growing global market, particularly around urbanized centers due to its relatively low cost and accessibility. Following the emergence of ASFV in China and subsequent spread throughout Asia, ASFV has become an issue of global concern leading to a number of national and international scientific conferences, workshops and specialized reports to address ongoing needs for detection and countermeasures to control and mitigate the impact of this health threat. The consistent conclusion is the need for a gap analysis of our current capacity to quickly respond to and effectively control an epidemic. There is an urgent need for harmonization, situation-adapted workflows, and basic knowledge of the disease itself.

This presentation is intended to consolidate and highlight the key issues detected during the most recent gap analyses of national and international ASF reference centers for diagnostics (USDA NVSL-NAHLN, GARA, and OIE/EURL), including a brief overview of FADDL activities in filling some of the gaps. The key message is expected to generate a good discussion of the necessity to orchestrate coordinated ways to reach shared research goals. Closing these gaps will require establishing secure ways to share data, enhance transparency, and work together in a more coordinated fashion.

Positive selection pressure on E2 protein of classical swine fever virus drives variations in virulence, pathogenesis and antigenicity: implication for epidemiological surveillance in endemic areas.

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Summary

Classical swine fever (CSF), caused by CSF virus (CSFV), is considered one of the most important infectious diseases with devasting consequences for the pig industry. Recent reports describe the emergence of new CSFV-strains resulting from the action of positive selection pressure, due mainly to the bottleneck effect generated by ineffective vaccination. Even though a decrease in the genetic diversity of the positive selected CSFV-strains has been observed by several research groups, there is little information about the effect of this selective force on the virulence degree, antigenicity and pathogenicity of this type of strains. Hence, the aim of the current study was to determine the effect of the positive selection pressure on these three parameters of CSFV-strains, emerged as result of the bottleneck effects induced by unproper vaccination in a CSF-endemic area. Moreover, the effect of the positive selected strains on the epidemiological surveillance system was assessed. By the combination of in vitro, in vivo and immunoinformatic approaches we revealed that the action of the positive selection pressure induces a decrease in virulence and alteration in pathogenicity and antigenicity. However, we also noted that the evolutionary process of CSFV, especially in segregated microenvironments, could contribute to the gain-fitness event, restoring the highly virulent pattern of the circulating strains. Besides, we denoted that the presence of low virulent strains selected by bottleneck effect after inefficient vaccination can lead to a relevant challenge for the epidemiological surveillance of CSF, contributing to under-reports of the disease, favoring the perpetuation of the virus in the field. In this study B-cell and CTL epitopes on the E2 3D-structure model were also identified. Thus, the current study provides novel and significant insights into variation in virulence, pathogenesis and antigenicity experienced by CSFV strains after the positive selection pressure effect.

"Evolution of porcine rubulavirus and control alternatives through recombinant immunogens". PhD. Julieta Sandra Cuevas-Romero

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El Rubulavirus porcino (PorPV) es el agente etiológico de la Enfermedad del Ojo Azul (EOA), la cual se considera una de las cuatro enfermedades más importantes que afectan la porcicultura nacional, ocasionando mortalidad en lechones y afección de los parámetros productivos y reproductivos en cerdos adultos. La enfermedad del ojo azul solamente ha sido reportada en México causando pérdidas económicas y problemas sanitario en la Industria porcina del país al convertirse en una limitante para su comercio nacional e internacional (APHIS, 2007). El impacto económico se asocia a la baja fertilidad y aumento en la presencia de mortinatos (19%) la presencia de momias (36%) disminución en el número total de lechones nacidos vivos (-4.1) y aumento de la mortalidad durante la primera semana de vida. Durante los últimos años se ha determinado que la EOA, se presenta como una enfermedad endémica en la zona oeste-centro del país, donde se estima una prevalencia del 9 al 23.7%, afectando aproximadamente un tercio del total de la población porcina nacional. Estudios recientes sobre la caracterización molecular del virus completo de diferentes aislamientos (2007 a 2013) colectados de nuevos brotes de la EOA, mostraron que existen diferentes variantes genéticas del Rubulavirus porcino, que podrían involucrar cambios en el tropismo, la patogenicidad, y la interacción del virus con el hospedero. No obstante, existen cepas virales que se han conservado por más de 25 años desde la presentación de los primeros brotes a principios de los 80's. Estos estudios indicaron la presencia de por lo menos tres variantes genéticas del PorPV que circulan en la población porcina de las áreas geográficas afectadas (centro del país).

Actualmente no existe un programa de vacunación oficial para prevenir esta enfermedad, de tal forma que cobra vital importancia el desarrollo de nuevos productos para su uso como posibles inmunógenos elaborados a partir de proteínas recombinantes. Por esta razón en el CENID-Microbiología del INIFAP, utilizando herramientas biotecnológicas relacionado a la expresión de genes, ha desarrollado un inmunógeno recombinante elaborado a partir de la proteína HN del Rubulavirus porcino (hemaglutinación-neuraminidasa) como el componente más abundante del virus y de mayor respuesta antigénica en animales infectados. Esta proteína HN, se ha expresado en el vector de Escherichia coli, como parte del concepto de "Vacunas de Nueva generación" la cual ha sido evaluada en condiciones experimentales obteniendo una adecuada respuesta inmune en hembras gestantes que provee una inmunidad pasiva a lechones vía calostro, determinada mediante pruebas de virus neutralización en cultivos celulares. Este producto recombinante se ha desarrollado como un candidato a vacuna en una plataforma biotecnológica económica y escalable para su producción en gran cantidad como un producto seguro y eficaz para prevención y control de la EOA, así como para el desarrollo de un sistema

diagnostico (DIVA) para futuros estudios y aplicaciones. Sin embargo, la estrategia más importante en el control de la EOA, representa las adecuadas medidas de higiene y bioseguridad en las granjas.

Presenter: Douglas P. Gladue

Title: Global African swine fever Research Alliance (GARA) Gap Analysis of Vaccines

Abstract

African swine fever virus (ASFV) causes a highly contagious disease called African swine fever (ASF), currently causing outbreaks in both Europe and Asia. This disease is often lethal for domestic pigs, causing extensive losses for the swine industry. Currently there is no commercially available treatment or vaccine to prevent this devastating disease, current outbreaks are controlled by culling of infected animals, and have already caused extensive losses to the swine industry in outbreak areas. Although some promising live-attenuated vaccines against ASFV have been published, there are several gaps in ASFV that are of great importance that need to be addressed before fully understanding the disease, and are needed to fully combat ASF. Recent developments in live attenuated vaccines for African swine fever are promising, the development and clinical results for these vaccines will be discussed

The Status of PRRSV in North America and the Influence of Vaccines

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The US Swine Pathogen Database (US-SPD) initiative, funded by the USDA-ARS-NADC-VPRU and National Pork Board of the US, has established a new and robust database of genetic sequences for significant swine pathogens. The US-SPD (https://swinepathogendb.org), is an open access web-based tool developed using the new USDA-ARS scientific high-perfomance computing cluster and network (SCINet) to assemble genetic sequence data, and link it with detection date and location, for the major pathogens of swine detected by the major US veterinary diagnostic laboratories. The laboratories include South Dakota Animal Disease Research & Diagnostic Laboratory (SDSU), Iowa State Veterinary Diagnostic Laboratory (ISU), Kansas State Veterinary Diagnostic Laboratory (KSU), and soon the Minnesota Veterinary Diagnostic Laboratory (MVDL). The database also contains over 12,000 PRRSV sequences collected primarily by SDSU and MVDL from 1989-2007.

The US-SPD presently houses porcine reproductive and respiratory syndrome virus (PRRSV), Seneca virus A, and swine coronaviruses, and will eventually include circoviruses and others, except for swine influenza A virus which is housed at the Influenza Research Database. Additional viruses, including foreign animal diseases (i.e., African swine fever virus) and major bacterial coinfecting pathogens are planned. The US-SPD has incorporated all available GenBank files that have been fully annotated, along with convenient tools to retrieve, display, and select sequences for further analyses. At present, the database includes almost 31,000 PRRSV sequences, 5000 PEDV, and 224 Seneca virus A sequences.

Using the US-SPD database all PRRSV nonstructural protein 2 (nsp2) and full-genome sequences detected around the world, including those detected by SDSU and ISU from 2014-2017, were assembled. In addition, all recent ORF5 sequences from SDSU, ISU and KSU were combined with a randomly selected set of 2000 GenBank ORF5 sequences and a 600-sequence reference gene set developed in 2010 describing PRRSV ORF5 diversity. We excluded high passage viruses, except for available vaccines, and synthetic sequences.

Nucleotide alignments were completed for each set of sequences, which numbered 775 genomes, 808 nsp2 and 4918 ORF5 regions. We then inferred the best-known maximum likelihood phylogenetic tree for each alignment. Two statistically supported genetic clades were detected for the genome and nsp2 trees. The first clade consisted mostly of older sequences, including all available modified live vaccines. The second clade represented the majority of recent isolates collected in the US. The ORF5 gene tree, made up of more than 6-fold more nucleotide sequences than that of the genome and nsp2 phylogenies, had three statistically supported genetic clades. For the major genetic clades, we implemented a Bayesian analysis to quantify the variation in evolutionary rate and used these data to identify genetic sites that are undergoing diversifying selection. Our analyses demonstrate the dynamic

landscape of PRRSV evolution with the cocirculation of multiple genetic clades, how the emergence of novel genetic types is associated with different evolutionary trajectories, and how data collated from all available data in the Western Hemisphere can be used to assess the potential efficacy of vaccine strains. These data demonstrate that PRRSV diversity is likely to limit the efficacy of current vaccines and the cocirculation of different genetic clades further generates heterogeneity among viruses that may emerge and spread in naïve host populations.

Senecavirus A: Research Update at NADC

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Senecavirus A (SVA) causes vesicular disease in swine that is clinically indistinguishable from foot-and-mouth disease (FMD). Therefore, when a vesicle is observed, a foreign animal disease investigation must be performed by trained personnel to rule out the presence of FMD virus. Conducting these investigations has economic impact for federal and state governments as well as for pork producers and slaughter plant facilities since they cannot move animals with active lesions until FMD is ruled out. In the US, vesicular disease investigations at some sow slaughter plants has become a weekly, and at times, a daily occurrence. These investigations have not detected FMD virus, but they have routinely detected SVA indicating this virus is responsible for significant economic loss for the affected slaughter plants and related swine herds. A potential solution to this problem is the use of SVA vaccination that would prevent clinical disease and thus reduce the number of vesicular disease investigations. This research update will cover current SVA vaccine research and pathogenesis studies at NADC.

Presenter: Oliver Lung

Title

High throughput sequencing of known, novel and unexpected viruses at the National Centre for Foreign Animal Disease, Canadian Food Inspection Agency

Abstract

Novel, emerging/re-emerging and exotic infectious diseases represent a growing challenge for both public and animal health authorities worldwide. The Canadian Food Inspection Agency's National Centre for Foreign Animal Disease (NCFAD) located in Winnipeg, Manitoba is a laboratory mandated with surveillance, diagnostic testing, diagnostic test development and scientific advice regarding high consequence infectious diseases related to animals. High-throughput sequencing (HTS) is becoming established as a powerful tool for pathogen identification, discovery and characterization. This presentation will include an overview of the new and only Canadian containment level 3 (CL3) HTS sequencing facility, ongoing activities related to the development of methodologies used for sequencing and bioinformatics analysis of priority viruses, as well as unknown or unexpected pathogens. Evaluation of user-friendly novel technologies for genome detection of livestock viruses will also be presented.

Foot and Mouth Disease in Swine

Carolina Stenfeldt, Jonathan Arzt, Teresa DelosSantos, Elizabeth Rieder, James Zhu and Luis Rodriguez

Pigs are highly susceptible to all serotypes of foot-and-mouth disease (FMD) virus with clinical signs ranging from vesicular disease to death due mainly to cardiopathy. Although clinical signs resemble those observed in ruminants (i.e. vesicular lesions in mouth and feet) the pathogenesis of FMD in pigs differs from that in ruminants. Unlike ruminants, pigs do not support persistent infection of FMDV and infectious virus is cleared within 28 days after infection, only some viral RNA can be detected beyond that point and disappears by 100 days. The primary site of viral replication in pigs is also different from that of ruminants with oral-pharyngeal tissue playing a major role in the pre-viremic subclinical phase of infection (as opposed to the nasal-pharyngeal tissue in cattle). There are also common features in FMD pathogenesis between pigs and cattle, such as the target cells in both species being primarily keratinized epithelial cells. Traditionally pigs have been considered as amplifiers of the FMD infection, although important aspects of the infection dynamics in pigs were not clear. Recent studies at PIADC addressed two important aspects; a. the temporal infectivity of pigs and b. the susceptibility of pigs to infection by contaminated feed. We will describe experiments showing that pigs can serve as source of infection to other pigs at least 24h before clinical signs are present and they are susceptible to infection by contaminated food. This information is relevant to the development of more accurate models for FMD transmission during outbreaks. Finally, we will discuss inactivated virus and vectored subunit vaccines, including the new leaderless FMDV 3B3D and Ad5-FMD vaccines and their potential use during control of FMD outbreaks in non-endemic settings.

Improved vaccine strategies to control swine influenza A virus

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Because of its plasticity and complicated epidemiology in swine, influenza A virus (IAV) presents a challenge to control through vaccination. There are intricate immune responses to IAV exposure, primary and repeated exposures in a lifetime, as well as responses to vaccines administered to immune and partially immune individuals. However, many of these types of studies are lacking in the swine host. Some experimental infection studies have shown cross-protection between influenza viruses even in the absence of cross-reactive serum hemagglutination inhibition (HI) antibodies. The immune mediators of this “broad” protection need further study, but may include a combination of mucosal IgA antibodies, cell mediated immune (CMI) responses, and cross-reactive neutralizing and/or neuraminidase inhibition (NI) antibodies. Whole inactivated vaccines (WIV) are administered parenterally through intramuscular routes whereas live attenuated influenza vaccines (LAIV) are typically delivered mucosally through intranasal routes. Vectored or RNA vaccines may be delivered through either route. The route of administration as well as the manner in which vaccine antigens are presented to the immune system influences the resulting host immune response. The immune response after intramuscular administration of WIV is fundamentally different from live virus infection. Vaccination immunity from WIV relies largely on inducing high titers of serum HI and neutralizing antibodies to the HA of the vaccine strain(s). In contrast, mucosal IgA antibodies or T-cells are not efficiently induced by WIV. In LAIV studies, cross-protection within a subtype has been shown to provide broader heterologous protection, but cross-protection between H1 and H3 IAV subtypes was limited. Vectored vaccines may induce a more balanced humoral and CMI responses, but do not typically stimulate the mucosal immune system if delivered by peripheral injection. Improved knowledge of protective responses at the respiratory mucosa and how different IAV vaccine platforms and viral antigens alter these responses are needed in swine to improve our ability to control this important pathogen that affects so many species.