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PROCINORTE

Animal Health Task Force Report

October 2019

Animal Health Task Force

Dr. Cyril G. Gay, Chair, USDA-ARS, United States

Dr. Elizabeth Loza-Rubio, CENID-Microbiology, INIFAP, Mexico

Dr. Jose Lopez, CFIA, Canada

Executive Summary

This year the members of Animal Health Task Force of PROCINORTE met in Mexico City to discuss the timely subject of Emerging Swine Pathogens that Pose a Threat to North America. Canadian, Mexican and U.S scientists, representing the Canadian Food Inspection Agency (CFIA), the United States Department of Agriculture's Agricultural Research Service (USDA-ARS), the Instituto Nacional de Investigaciones Forestales, Agrícolas y Pecuarias (INIFAP), including other government agencies and universities from North America, met in a spirit of collaboration to share their experiences in this important emerging issue. This year, in addition to the North American participants, the Caribbean Animal Health Network (CaribVet), was invited again in order to share experiences of mutual interest in this important area of animal health in the region and to discuss possible collaborative initiatives. Importantly, for the first time, a representative from PROCISUR participated, which was critical with the realization that disease outbreaks with dangerous pathogens like African Swine Fever could first enter through South America. The workshop brought together a total of 37 participants: 20 from Mexico, eight from the United States, three from Canada, one scientist representing CaribVet and five representing other organizations. Scientific exchanges and training has been provided and will continue for INIFAP scientists in laboratories in the United States and Canada.

Overview of PROCINORTE and the Animal Health Task Force

PROCINORTE is an important mechanism to facilitate the institutional and technical integration of the United States, Canada, and Mexico under the umbrella of the Inter-American Institute for Cooperation on Agriculture's (IICA) Northern Regional Center. PROCINORTE is a cooperative program in research and technology with four task forces (Animal Health, Plant Health, Tropical and Sub-Tropical Fruits & Genetic Resources) that determine common research priorities. Setting work priorities is a major task, because they are strategically important to guarantee the allocation and effective use of national and international resource availability, and they also are important in developing effective collaboration efforts to take advantages of the opportunities of multinational research, especially in those research fields where there is a need to optimize research resources, avoid duplication of efforts, and optimize the use of facilities, and equipment and to facilitate a broad environmental validation of results. PROCINORTE objectives are to:

1. Promote dialogue to identify priority research issues common to the three countries and to influence the regional, hemispheric and global agendas.
2. Facilitate the exchange of experiences, information and training through the building of linkages among public and private country institutions of the Northern region (PROCINORTE) and between the major research and technology transfer actors in the region, hemisphere and the world.
3. Facilitate the collaboration among the countries to solve problems of mutual interest.

Potential mutual benefits from collaborations include:

- a) Perform strategic research for agricultural development.
- b) Development of technologies for agribusiness benefit.

- c) Strengthen technological exchange.
- d) Development and use of methodologies for the establishment of standard norms for common use in commodities trade.
- e) Provide solutions to common problems and challenges to help the countries to cover their present population needs more efficiently.
- f) Develop scientific solutions to agricultural problems, to increase profitability for farmers preserving their land and natural resources.

The PROCINORTE Animal Health Task Force (PAHTF) is comprised of leading government scientists from the three member countries. The PAHTF meets face-to-face at least once a year by holding scientific workshops on animal diseases deemed to be priorities for North America. The primary goal of these workshops is to determine the disease situation and available countermeasures to control and respond to animal diseases that impact agriculture in the three member countries. The ultimate goal of the workshops is to provide scientists the opportunity to get to know each other and establish research collaborations that will advance research to enhance animal health in the three member countries.

The PAHTF identified the following criteria for prioritizing areas of research:

1. Diseases that impact the movement of animals between Canada, U.S, and Mexico.
2. Diseases that have significant economic and/or public health impact.
3. Diseases that are national priorities for Canada, U.S, or Mexico.
4. Diseases which are endemic in one or two of the three countries where the disease free-country will benefit from the expertise of the endemic country, including the ability to work directly with a foreign animal pathogen, expert scientists, and pathogen-dedicated facilities.
5. Areas of expertise in one country may complement expertise in another country enhancing the formation of multi-disciplinary research teams.
6. Opportunities to enhance the impact of limited financial resources.
7. Opportunities to develop control measures such as diagnostics and vaccines that can be applied within the three countries, resulting in uniform diagnostic tools and control measures.
8. Opportunities to bank and share samples for diagnostic validation and future research.

The expected results from the activities of the PAHTF include:

1. Sharing of research activities and potential changes in high consequence animal disease control program policies, procedures and techniques.
2. Coordination of research activities to promote collaborations between the US, Canada and México.
3. Development of an understanding of measures used in the US, Canada and México for the control and eradication of high consequence animal diseases and harmonization (where appropriate) of policies and procedures.

Research Collaborations

As a result of our previous workshops, the following collaborative PROCINORTE Research Projects are on-going:

- North American Assessment of Bluetongue and *Culicoides* Vectors
- Development of Strategies to Control Tick-Borne Babesial Pathogens of Livestock. Performance of New Reagents and Methods for the Diagnosis and Control of Equine Piroplasmiasis is a sub-project of the above project:
- Collaborative Research on Vesicular Stomatitis Diagnosis

The following PROCINORTE project has been completed and the final report was submitted:

- Use of IFN-expressing vectors in control of Nipah and Ebola virus diseases in swine

Training

As a result of previous workshops organized by the PROCINORTE Animal Health Task Force, the following scientific exchanges and training, among others, have taken place:

Mayra Cobaxin, Instituto Nacional de Investigaciones Forestales, Agrícolas y Pecuarias, INIFAP, México:

Training to acquire the tools for growing *Anaplasma marginale* in tick cell lines at the Animal Diseases Research Unit, Agricultural Research Service (ARS), USDA, Pullman, Washington.

Edith Roja-Anaya, Department of Biotechnology and Animal Health, INIFAP, Mexico: Training to initiate collaborative metagenomics research in small ruminants, National Animal Disease Center (NADC), Agricultural Research Service (ARS), USDA, Ames, Iowa.

Elizabeth Loza Rubio, Department of Biotechnology and Animal Health, INIFAP, Mexico: Training to sort trapped midges, CFIA Lethbridge Laboratory, Alberta, Canada.

Based on research collaborations that come up as a result of our recent workshop discussions and networking, more scientific exchanges and training visits for Mexican scientists in Canadian and US laboratories will most likely occur.

Outreach

Students

The workshops organized by the PROCINORTE Animal Health Task Force have included graduate students from Mexico and provided opportunities for official presentations and

interactions with leading scientists in their field from Canada, Mexico and the United States of America. Opportunities for training and exchange are also considered when the budget provided by the PROCINORTE Executive Committee allows.

Caribbean Animal Health Network

The Caribbean islands consist of 34 countries and territories. Animal production is an important industry that is critical to the economies of the island nations and key contributors to food security. Importantly, the Caribbean is geographically part of North America with the Caribbean islands bordering the United States and Mexico. As such, the emergence of new infectious diseases in the Caribbean with its tropical climate poses a significant threat to North America and the PROCINORTE Animal Health Task Force has reached out to the Caribbean Animal Health Network – CaribVet <https://www.caribvet.net/>

CaribVet provides strategic opportunities for research collaborations that are likely to help protect animal agriculture in North America against the threat of emerging pests and infectious diseases that are present in the Caribbean. A good example is the Tropical Bont Tick, which is also the tick vector for Heartwater, an important disease of ruminants in Africa and some of the Caribbean islands. Strategic opportunities for collaborations with CaribVet include:

- Epidemiological surveillance, control and eradication of diseases
- Identification of pathogens
- Prevention and emergency preparedness plans for priority diseases

Accomplishments in the last two years include the participation of CaribVet at the annual PROCINORTE Animal Health Task Force workshop. Participation from CaribVet at the workshops provided a forum for scientific exchanges and areas of common interest for research projects that are relevant to North America.

Workshop

This year, the following topics were the subject of our workshop on “Emerging Swine Pathogens that Pose a Threat to North America,” September 9-10, 2019 – Mexico City, Mexico

The following three areas were the focus of the workshop:

- Epidemiology
- Diagnostics
- Countermeasures

Numerous presentations and discussions regarding the above took place on the following swine diseases:

- African swine fever (ASF)
- Foot-and-Mouth Disease
- Classical Swine Fever (CSF)
- Influenza A
- Seneca Valley Virus
- Rubulavirus
- Porcine Reproductive and Respiratory Syndrome Virus (PRRSV)

Presentations dealing with potential funding sources and collaborative research options were discussed, including:

- Inter-American Institute for Cooperation on Agriculture (IICA)
- PROCINORTE
- PROCISUR
- CaribVet
- Foundation for Food and Agriculture Research (FFAR)
- STAR-IDAZ
- Global African Swine Fever Research Alliance (GARA)

Agenda

Monday, 9 September 2019

09:00-09:10 **Welcome**

Fernando de la Torre Sánchez, Director, INIFAP, Mexico
Dr. Diego Montenegro, IICA Representative in Mexico

09:10-09:20 ***Introductions, Purpose, Expected Outcomes***

Cyril Gay, USDA-ARS – TF Leader, USA
Elizabeth Loza-Rubio, INIFAP – TF Member, Mexico
Jose Lopez, CFIA – TF Member, Canada

Epidemiology

Chair: Elizabeth Loza Rubio, INIFAP

09:20-10:00 Elizabeth Loza Rubio, CENID-SAI, INIFAP

Cooperation between the Ministry of Agriculture and INIFAP: Historical study of swine influenza.

10:00-11:00 Roberto Navarro López. CPA-SENASICA-SADER.

Activities in Mexico for the prevention of African Swine Fever

11:00-11:30 Break

11:30-12:30 Kelly Lager, NADC, ARS, USDA

Seneca Valley Virus

12:30-13:30 Luis Rodriguez, FADRU, ARS, USDA

Foot-and-Mouth Disease

13:30-14:30 Lunch

Diagnostics

Chair: Jose Lopez, CFIA

14:30-15:30 Consuelo Carrillo, FADDL, APHIS, USDA

Global African Swine Fever Research Alliance (GARA) Gap Analysis of Diagnostic Tests.

15:30-16:30 Aruna Ambagala, CFIA

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

16:30-17:30 Oliver Lung, CFIA

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

[Tuesday, 10 September 2019](#)

Countermeasures

Chair: Cyril Gay, USDA-ARS

09:00-09:50 Sandra Cuevas Romero, CENID-SAI, INIFAP

Evolution of porcine Rubulavirus and control alternatives through recombinant immunogens.

09:50-10:40 Amy L. Vincent, NADC, ARS, USDA

Improved vaccine strategies to control swine influenza A virus.

10:40-11:00 Break

11:00-11:50 Manuel Borca, FADRU, ARS, USDA

FlagT4 Classical Swine Fever Vaccine

11:50-12:40 Liani Coronado Báez, CENSA, Cuba

Positive Selection Pressure on E2 Protein of CSF Virus Drives Variations in Virulence, Pathogenesis & Antigenicity: Implications for Epidemiological Surveillance in Endemic Areas

12:40-13:40 Lunch

13:40-14:30 Doug Gladue, FADRU, ARS, USDA

Global African Swine Fever Research Alliance (GARA) Gap Analysis of Vaccines

14:30-15:20 Kay Faaberg, NADC, ARS, USDA

Porcine Reproductive and Respiratory Syndrome Virus (PRRSV) vaccines

Support and Potential Opportunities for Research Collaborations in the Americas

15:20-17:00 Chair: Armando Mateos, Animal health specialist, IICA Mexico

Inter-American Institute for Cooperation on Agriculture (IICA)

Horrys Friaca, IICA's Agricultural Health Program

PROCINORTE

Jose Lopez, CFIA, Canada

PROCISUR

Ariel Pereda, INTA, Argentina

CaribVet

Jennifer Pradel, CIRAD, Guadeloupe, France (Invited)

Foundation for Food and Agriculture Research (FFAR)

Tim Kurt, FFAR, U.S

STAR-IDAZ

Francisco Suárez Güemes, Director, Facultad de Medicina Veterinaria y Zootecnia
Universidad Nacional Autónoma de México

Global African Swine Fever Research Alliance (GARA)
Cyril Gay, Executive Committee, GARA

Abstracts

COOPERATION BETWEEN THE MINISTRY OF AGRICULTURE AND INIFAP: HISTORICAL STUDY OF SWINE INFLUENZA.

Elizabeth Loza-Rubio
Edith Rojas-Anaya

INIFAP

In Mexico, the interinstitutional work plan was developed in July 2009 between Ministry of Agriculture and INIFAP in order to understand the outbreak produced at that year. The plan considered, among others, the Multisectoral Operating Strategy, which represented its translation into the specific actions to be developed by each institution before the probable emergency of a new pandemic. In Ministry of Health, on April 13, 2009 in the capital city of Oaxaca, a southern state, a 39-year-old female died of severe atypical pneumonia. A local laboratory diagnosed a coronavirus as a causative agent, leading health authorities to send the sample to the United States Centers for Disease Prevention and Control (CDC). Later, when additional cases of atypical pneumonia were reported, samples were shipped to the Winnipeg laboratory in Canada. The influenza continued to behave atypically and, 5 days after the epidemiologic alert, the necessary steps were taken to prevent the population from attending crowded places, and the use of the "etiquette sneeze" and frequent hand washing were recommended as preventive measures. On April 23, the laboratory tests fully identified the virus as influenza A(H1N1) from a virus strain unknown until then, which meant that its behavior, virulence, transmission capacity and origin were all unknown. And initially, even its susceptibility to the available antivirals, the magnitude of the associated risk and its pandemic potential were also unknown. The previous experience of other influenza pandemics in other countries was the only source of knowledge. In July 2009, Ministry of Agriculture and INIFAP considered to begin a study for determining the real role of swine in that pandemic, since huge economic losses were produced because of lack of trade.

The influenza virus infection in pigs is considered an important public health concern, therefore, the aims of the study were to isolate and characterize viruses from Mexican pigs in the Bajío, as well as, determine seropositive frequencies and risk factors associated with the spread of swine influenza virus (SIV). Serum samples from 2048 pigs of 256 farms were analyzed for detection of antibodies against SIV subtypes

H1N1 and H3N2, and H1N1/2009 pandemic. Nasal swabs were collected to virus identification by real time reverse transcription-PCR. To evaluate the potential risk factors associated with SIV, were analyzed using X2 test and logistic regression model. A virus isolated from Guanajuato was characterized and shown to be H1N1. Several risk factors were associated with seroprevalence of SIV: breeding stock and weaning pigs, the boars, infected animals, over the road farms, waste feeding, wetlands presence, pigs breeding by swine workers and breeding-fattening pig farms. In conclusion, the virus A/cerdo/Mexico/Gto_Sal_1/2011 (H1N1) isolate indicates to be swine origin and not related with human or avian sequences. This isolated shown high homology with other Mexican isolates, mainly because of trade, also, there was a high seroprevalence of H1N1 subtype bringing on because of the significative risk factors found in this study.

Some lessons learned are: 1) The coordination among all the government levels was achieved mainly through the States' Secretaries. 2) The implementation of preventive measures that precluded a major contingency from occurring was possible thanks to the maturity with which all Mexicans behaved 3) The presence and solidarity of international organizations, foreign governments and private businesses provided the technical support that facilitated both confirming the presence of a new virus and the access to the scientific knowledge accrued internationally. 4) The rest of the world benefited from the Mexican experience thanks to the timeliness with which the government issued the sanitary alert and informed about the course of the epidemic, 5) The knowledge shared by Mexico provided the countries who have faced the human influenza virus A(H1N1) with information about its epidemiologic characteristics, its clinical picture and the chance of cure if timely healthcare is provided, and 6) The emergence of the new influenza virus will undoubtedly become a public health landmark and will be recorded in the annals of world history.

SENECAVIRUS A: RESEARCH UPDATE AT NADC

Kelly Lager and Alexandra Buckley

Virus and Prion Research Unit, National Animal Disease Center, USDA-ARS, Ames, IA, USA

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.

FOOT AND MOUTH DISEASE IN SWINE

Carolina Stenfeldt,
Jonathan Arzt,
Teresa DelosSantos,
Elizabeth Rieder,
James Zhu
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.

ASF DIAGNOSTICS GAP ANALYSIS: GARA AND EURL/OIE VIEWS AND RESULTS

Consuelo Carrillo

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 health, 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.

ASF AND CSF DIAGNOSTICS AND RESEARCH AT THE NATIONAL CENTRE FOR FOREIGN ANIMAL DISEASE, CANADIAN FOOD INSPECTION AGENCY

Aruna Ambagala
National Centre for Foreign Animal Disease (NCFAD),
Canadian Food Inspection Agency,
Winnipeg, Canada

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.

HIGH THROUGHPUT SEQUENCING OF KNOWN, NOVEL AND UNEXPECTED VIRUSES AT THE NATIONAL CENTRE FOR FOREIGN ANIMAL DISEASE, CANADIAN FOOD INSPECTION AGENCY

Oliver Lung
National Centre for Foreign Animal Disease (NCFAD),
Canadian Food Inspection Agency,
Winnipeg, Canada

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.

EVOLUTION OF PORCINE RUBULAVIRUS AND CONTROL ALTERNATIVES THROUGH RECOMBINANT IMMUNOGENS.

PhD. Julieta Sandra Cuevas-Romero
National Center of Animal Health and Inocuity
INIFAP

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 diagnóstico (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.

IMPROVED VACCINE STRATEGIES TO CONTROL SWINE INFLUENZA A VIRUS

Amy L. Vincent DVM, PhD

Virus and Prion Research Unit, National Animal Disease Center, USDA-ARS, Ames, IA,
USA

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.

STATUS OF CSF RESEARCH AT ARS TOWARDS THE DEVELOPMENT OF NOVEL MARKER VACCINES

Manuel V. Borca

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.

POSITIVE SELECTION PRESSURE ON E2 PROTEIN OF CSF VIRUS DRIVES VARIATIONS IN VIRULENCE, PATHOGENESIS & ANTIGENICITY: IMPLICATIONS FOR EPIDEMIOLOGICAL SURVEILLANCE IN ENDEMIC AREAS

Liani Coronado Báez,
Centro Nacional de Sanidad Agropecuaria (CENSA), OIE Collaborating Centre for
Diagnosis and Risk Analysis of the Caribbean Region, La Habana 32700, Cuba.

Classical swine fever (CSF), caused by CSF virus (CSFV), is considered one of the most important infectious diseases with devastating 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 improper 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.

GLOBAL AFRICAN SWINE FEVER RESEARCH ALLIANCE (GARA) GAP ANALYSIS OF VACCINES

Douglas P. Gladue

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-performance 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.